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China looks West

ALL our news pages this week are devoted to Joseph Needham's impressions of developments in science policy in present-day China. Dr Needham, of course, needs no introduction as a renowned historian of Chinese science and technology. His recent month-long visit to China was made with encouragement and support from *Nature*; we wished to learn of the impact of recent political events on science, and assess the results of the National Conference on Science Policy held earlier this year.

It is clear that Chinese scientists are living through times of immense change—of a sort that Westerners would find almost impossible to comprehend. Within the past few years kept firmly in their place—even actively discouraged—by a tide of anti-intellectualism, they now find themselves given every support, and treated as a precious resource. Any doubts about the seriousness of this new resolve must be dispelled by the massive educational exchange programmes that the Chinese are negotiating with scientifically advanced countries.

So what do Chinese scientists need most at present? They certainly need a period of many years of tranquility, away from the limelight of political attention. For a generation science and higher education in China have lived on a switchback of alternating support, indifference, and even open hostility. Science cannot survive intact given such a bumpy ride. However, given its tranquility, it would be a pity if Chinese science and technology were to adopt the western model entirely without criticism. For while China has much to learn from the West in terms of building up its capacity to perform basic research, it also has extensive experience in applying science in ways that, while profoundly different from the West—may be becoming increasingly relevant on a global scale.

Certainly the Chinese are putting much thought into the most appropriate form of scientific and technological infrastructure; but they are looking to the West for advice. For example, a group of middle-level scientific managers is shortly to make a month's study visit to the US organised by the National Academy of Sciences, and will be making similar visits to other western countries to compare different ways of organising scientific effort. Further, despite the continued lack of full diplomatic relations with the US, economic interest appears to have taken over command from ideological differences in determining the scientific and technological relations between the two countries.

Indeed, the apparent non-political nature of science and technology has made contact and co-operation in these areas a convenient way of opening up broader social and economic contact while avoiding the embarrassment caused by the fact that the US still keeps an ambassador in Taiwan, but has only a liaison mission in Peking.

The most significant manifestation so far of the new situation was the visit of a 14-person delegation of top US science policy-makers, headed by the President's Science Adviser Dr Frank Press and including the heads of the National Science Foundation, the National Aeronautics and Space Administration, and the Department of Energy's

Office of Energy Research, to Peking last month for three days of talks.

Although no specific agreements were reached during these discussions, they helped create a broad framework within which more detailed discussion on specific areas are now taking place. For example, some hundreds of educational exchanges are likely to be set up and in October energy secretary Dr James Schlesinger will be visiting China to discuss, among other things, US involvement in developing China's oil reserves. Here the Chinese are interested in using US know-how to help exploit oil deposits off the coast as a way of financing their ambitious industrialisation programmes. US oil companies see a potential investment of between \$25 and \$50 billion in shared development programmes, which themselves could open up China to broader economic relations with the western world.

Several observers have drawn attention to parallels between China's relatively privileged position—as a relative late-comer to western-style industrialisation, able to be highly selective in the science and technology that it imports—and Japan fifty years ago.

However, with the yen now driving the dollar into the ground on international currency markets, the potential economic challenge has not gone unnoticed in US industry. And at the same time as it opens up the catalogues of the machine tool manufacturers, the US Department of Commerce is also providing instructions in US export controls.

Another problem facing the world will be the environmental and ecological stresses that could result from China reaching the level of industrialisation of western nations. China contains a quarter of the world's people, and it is estimated, for example, that at current western levels of oil consumption, China has the capacity for consumption twice the total annual production of the OPEC countries. And again one has only to look at the Japanese experience to find illustrations of the dangers that can accompany the application of technology with insufficient consideration of its side-effects.

In recent years, many in the West have turned to China as providing, under Chairman Mao's guidance, a different approach to ways of applying science, in which meeting basic human needs has often seemed to take precedence over the pursuit of economic profit. Recent travellers to China have reported that although many members of the new leadership are keen on pursuing the western development model, and are developing their scientific activities within this framework, some of their younger colleagues are giving this model close scrutiny.

From the West, one can only wait and see which way the political winds will turn. Certainly there is much to applaud in the concerted effort that the Chinese are now applying to building up the necessary scientific and technological infrastructure for science to play an active role in the nation's development. But one hopes that, in the process, the Chinese will be sufficiently discriminating and selective to preserve what is best of both eastern and western traditions. □





Science reborn in China

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Hsinhua

Special report by Dr Joseph Needham

Rise and fall of the anti-intellectual 'gang'

TOGETHER with my chief collaborator, Dr Lu Gwei-Djen, I spent more than one month in the People's Republic of China this last April and May, making a special study of the present situation of science, technology and medicine among the Chinese people. Our interest on this fifth visit centred on the important National Science Policy Conference which took place in Peking last March, and it is important that this conference, symbolising as it did a complete change in the climate of science, technology and medicine in China, should be brought to the attention of the readers of *Nature**.

However, the conference cannot be understood at all without some comprehension of the political situation in the country under the group commonly called in China the "Gang of Four" (*ssu jen pang*). Although the term "group" would be the normal expression in English, and "cabal" the best one, we may conveniently use in what follows the brief and objective abbreviation G₄. They exercised a dominant influence in China in the later stages of the Cultural Revolution during the last years of Chairman Mao (1974–76) and were overthrown in October of the latter year.

Of the members of the group, the only one well-known in the West was Chiang Ch'ing, the estranged third wife of Chairman Mao, herself a former Shanghai actress, and for some time prominent in cultural matters. She was associated with Chang Chün-Chao, a theoretician, Yao Wên-Yuan, a propagandist in control of the mass media including all press and radio, and Wang Hung-Wên, once a security guard in a Shanghai textile mill. Their activities

have been greatly misunderstood in the West; 'women's liberation', for instance, had little to do with it. But they certainly propagated a whole range of 'ultra-leftist' attitudes, demands and practices; and the universal view in China today is that they did this essentially in pursuit of personal power, ignoring the absurdities into which their followers were led. By a pendulum-swing effect, ultra-leftism had originally been a reaction against the clear rightism of Liu Shao-Ch'i, one time head of state and patron of a technocratic and bureaucratic élitism, who fell from power in 1967. There was little to distinguish the ultra-leftism utilised by Lin Piao, army general chosen by Mao as his successor, but later the leader of an unsuccessful coup, and his group, from that of Chiang Ch'ing and hers. However, they could not get on personally, so the latter cabal took over after the former came to a stop with Lin Piao's flight and death in 1971, though there was little to distinguish one form of power-seeking from the other.

Looking back, there can be no doubt that the activities of G₄ should be called a veritable heresy of Maoist ideology, analogous to some of the heresies which afflicted the Christian church in earlier centuries. I should not hesitate to call it a *cultus ignorantiae*, quite similar in tendency to movements which have been known in European history. One thinks of certain Gnostic sects, or the persecution of the Neo-Platonists by the monks of Cyril of Alexandria in the early fifth century, or the spiritual Franciscans, or some of the mystical doctrines of the Middle Ages. The Protestant tradition could also show much anti-intellectualism, as in the ideas of John Wyclif and William Dell.

In fact G₄ were fundamentally anti-intellectual, and inimical to scientists

and technologists in particular. They built upon the most narrow-minded backwoods prejudices of rural China, almost recalling the early opposition to railways and telegraphs on geomantic grounds (*fêng shui*) at the beginning of this century. As Chairman Hua said in his speech at the conference, these people maintained that the more knowledge and skill a man had, the more reactionary he would be likely to be, and one of their slogans was that "it is preferable to have workers with no culture at all"—so long as their ideas on the class-struggle agreed with the orthodoxy propagated by G₄. They went so far as to say that "knowledge is an aspect of private property and capitalism", so that China could face the world better with no modern science and only traditional technology.

Under their rule there was a list of "nine sorts of evil people". The first five had been defined long ago, in the heyday of Chairman Mao, as (1) landlords, (2) rich peasant-farmers, (3) counter-revolutionaries, (4) hooligans, vandals and criminals, (5) rightist politicians. Then under the Cultural Revolution were added (6) national traitors, (7) spies, (8) capitalist sympathisers or theorists; but it took G₄ to add the intellectuals and scientists as what they called "the stinking ninth category". People in China today have no hesitation in telling one that G₄ brought the economy to the brink of economic collapse. Exactly why will presently appear. To sum it up, the group would certainly have agreed with the words of that politician spoken at the time of the death of Lavoisier—"La Révolution n'a pas besoin des savants"—but they probably never knew them.

It might well be asked whether there

Above left, the author and Dr Lu Gwei-Djen; above right, Vice Premier Fang Yi greets scientists.

*The position in the latter part of last year was described by M. B. Bullock, "Science in Hua's China: the Key to Modernisation", *Nature* 270, 462; 1977.

could have been any connection with this anti-scientific wave in China and the anti-scientism of the counter-culture in the West? Was there concern about the danger of pollution of the environment, or anything similar to the strong feeling in the West against nuclear power and nuclear weapons? The answer is that there was no connection. In China people had hardly heard of these things; the phenomenon was quite different and *sui generis*. Naturally it takes great mental effort to respond to the call for modernisation of Chinese society, and the achievement of equality with the advanced industrial world by the end of the present century, so G₄ saw that they could build upon the natural lethargy of the masses faced with this great but necessary 'call to arms'. It was a kind of isolationism, almost a tendency to 'hide one's head in the sand'.

The evil effects of G₄'s activities were rather patchy, particularly bad in the northern provinces such as Liaoning, and the western ones such as Szechuan, Kansu and Kweichow; paradoxically bad in highly industrialised Shanghai, no doubt because this was the personal base of Chiang Ch'ing and Wang Hung-wên. One of the worst incidents took place at the National Institute of Optical and Precision Machinery at Ch'ang-Ch'un in Kirin province, where an individual named Shan Kuei-Chang succeeded in arresting a couple of hundred of the scientists and technologists. Some of them suffered torture in prison and others were driven to commit suicide. After the downfall of G₄, careful investigation revealed that all the charges of spying etc. were spurious, and everyone was rehabilitated, while Shan Kuei-Chang was turned over to the Department of Justice—but the setback to the work of the Institute (and indeed science in the north in general) can easily be imagined¹. Our experience was that there were great variations in the extent to which G₄ ruined the work of scientific institutes. For example, the National Tropical Products Research Institute on Hainan Island seemed to have been very badly affected, while on the other hand the Huanan Agricultural Institute and College at Kuangchow (Canton) had been able to carry on excellent research and teaching, even in such non-practical subjects as the history of agriculture, throughout the period.

Some of the worst effects of G₄'s depredations on science and industry occurred in two linked ways, their insistence on immediate practical results, and their extremely anti-foreign prejudice. The famous slogan, *tzu li kêng shêng* (improvement of livelihood by one's own efforts alone), coined first eighteen years ago when the Russians

suddenly withdrew from China all their technicians, could easily be distorted into a rigid xenophobia. For example, shipbuilding in Shanghai was quite disrupted for some years because even when only ten per cent or less of the equipment needed to finish a ship was required from abroad, importation was forbidden, and production languished. So also, the twisting of this slogan hit all laboratory work, because the import of any piece of apparatus was thwarted, even when preparations were far advanced in China for making it there.

Similarly, G₄ were against the learning and teaching of foreign languages, so that in the medical world, for example, courses for foreign physicians with modern-Western training could only begin after the overthrow. Again, the emphasis on 'autarky' produced lamentable results in Hainan Island. G₄ had most of the coffee plantations uprooted in the interests of intensifying rubber production, but they urged this on in an unreasonable way; they insisted on tapping twice a day, though it ought to be only once every second day, and also when it rained, though this is known to rot the tree. All the experts were sent away, and the island is only now recovering. Similarly, in the neighbourhood of Shanghai there was the erroneous theory that all rural communes should be autarkic, with the results that they took to growing grain and for several winters vegetables were almost unobtainable in the city of Shanghai.

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The T'ang-shan earthquake: was it predicted?

The cutting off of funds for all the higher research institutions was, under G₄, a matter of course, and the completion of the magnificent new building of the National Institute of Biochemistry in Shanghai was delayed for at least a couple of years. Necessarily in alliance with this was a general despising of science and scientists. Here one of the most remarkable stories which reached us concerns the terrible earthquake which caused so much damage and loss of life at T'ang-shan. Some of our informants were themselves in the Liaoning Seismological Station the day before that earthquake, and they saw for themselves evidence of earth movements. These were, it is said, reported hour by hour to Peking, but the top level prevented any action being taken. When the earthquake came, Chairman Hua flew immediately to the spot by helicopter, and acquired great merit among the people, but the cabal are said to have laughed it off, saying that the death of millions was a small matter in comparison with moral, anti-intellectual orthodoxy. We learnt further that the reports from seven seismographic stations were ignored, not only because of the atmosphere at 'cabinet level' but because the cabal had a following within Academia Sinica itself of suddenly upgraded people who failed to pass all the warnings on. In a way it is a matter of congratulation for Chinese seismology to know that the T'ang-shan earthquake was in fact to some extent predicted, and that it was only political circumstances which prevented any action being taken.

Other policies of G₄ involved exaggerating the time spent on manual work and political discussion in schools and universities, including totally impractical ideas such as 'the university in the fields' and 'open-door research'. *Hsia fang* (rustication), the sending of students, intellectuals and administrators to do manual work in farm or factory for a while, has long been a very important concept in Chinese society. Of course, there is no reason to be against the idea of one or two years' national service in industry or agriculture before entry to the universities, nor need one look unfavourably upon the periodical return of office-workers and intellectuals to practical activities of various kinds. Essentially these were good ideas; but G₄ used *hsia fang* as a veritable punishment for intellectuals and scientists. One venerable pathology professor was supposed to go to lecture on carcinogenesis to medical students while they were picking cotton. The Director of a world-famous Physiological Institute was sent out to 'cure low back pain in peasant-farmers', and the Vice-Director of a leading Biochemical Institute was

dispatched to 'solve plant virus problems in the commune itself' without any laboratory facilities. Many people who had recently been students told of the exaggerated time spent on political discussion in colleges. But all this has now been quite rectified, as we shall see.

The undermining of China's heavy industry came about largely through the promotion of the wrong personnel. For example, near Kweiyang there is a large cement factory, but G₄ caused such disruption in it that it went out of production entirely for about four years. If social or ethical orthodoxy is taken as the only criterion for promotion, and technological skill ignored, machines will just break down, and this is what happened all over China. Planning was reduced to chaos; for example, the Kweiyang calamity com-

pletely inhibited the construction of a large new hydro-electric plant in the gorges of the Wu Chiang river.

Many other interesting points would demand attention here if there were room. The cabal was against not only heavy industry, high technology, and advanced science, but also against the modernisation of the army, and indeed, all modernisation. As Premier Têng has said, G₄ went to the absurd length of claiming that "if the Four Modernisations are carried through, the restoration of capitalism will happen on the same day". They and their followers interfered everywhere in museums, attacked archaeology as a great branch of culture (partly no doubt because the late Premier Chou Ân-Lai strongly supported it) and were largely responsible for the anti-Confucius movement and the over-estimate of the Legalists

in Chinese history. *A fortiori*, they were against the history of science, technology and medicine, but these have made a happy revival since their overthrow.

Everywhere we went in China, we found a buoyant atmosphere, full of confidence for the future as a result of the great political changes of the past two years. Everyone speaks freely of the "springtime of science", of a "new Long March" towards becoming an advanced scientific country², even of a "new Liberation". The National Science Policy Conference was the guarantee and seal of all this; it will be the subject of the following article. □

1. (There are accounts in *New China News Agency Bulletin*, 30 April, p 11; and *Peking Review*, 1978, 21 (no 19), 12 May.)

2. (*Peking Review* 21 (no 14), 3; 1978).

Springtime for the people, springtime for science

FROM 17 to 27 March, 6,000 scientists met in conference in Peking to take decisions about the science policy of the Chinese nation for many years to come. These taking part ranged from young "barefoot seismologists" to veteran scientists, many of whom had been brought back from retirement. As one of the leading professors of Peking University put it to us when we arrived a month later, the fundamental effect of the conference had been a liberation of thought and confidence on the part of all scientific personnel, whether concerned with pure or applied science. And it seems that the long-enduring mistrust of foreign-trained and western-educated senior scientists is now at last abating.

The followers of the Cabal of Four (G₄) had constantly said that scientists were not really workers but middle-class self-seekers who used their knowledge for personal advancement alone and lacked all public-spirited conscience that science and technology should be at the service of the people as a whole. The conference was a decisive rebuttal of this point of view. It also prepared and accepted plans for developing research activities during the coming eight years, and indeed until the end of the century when the intention is to have caught up with world science. The third major topic of the conference was how the Communist Party could help scientists. The insistence of G₄ on immediate practical results was recognised as hopelessly wrong. All the funds for research which had been cut off were now restored, and indeed greatly increased. Furthermore, while dialectical materialism was accepted as the most satisfactory world-outlook, there was

a recognition that it could never substitute for the direct planning of scientific research, as had been the belief of some philosophers associated with G₄.

The President of the National Academy, Academia Sinica, Kuo Mo-Jo, the veteran archaeologist and ancient historian, poet, playwright and statesman, insisted on coming to the conference from his hospital sick-bed to deliver a stirring speech; since then he has in fact died, greatly mourned. He recalled the frustrating experiences of the early generations of modern scientists in China during the warlord and Kuomintang periods, contrasting it with the great hopes legitimately entertained by the Chinese scientists after the triumph of the Party in the Civil War. Chairman Mao and Premier Chou at that time drew up a splendid blueprint for transforming China into a modern, powerful, socialist country, and they showed the most thoughtful solicitude for all scientific undertakings and scientists. Then, unfortunately, there arose during the last years of Chairman Mao, the anti-scientific movement of G₄, but now this had been overcome. Kuo urged the scientific workers of the younger generation to be the pioneers in catching up with, and surpassing, the world's most advanced scientific levels. And it was Kuo Mo-Jo who coined the phrase "springtime for the people, springtime for science". He quoted the eighth-century poet, Pai Chü-I: "When the sun comes forth, the crimson flowers by the riverside look more fiery than flame; when spring sets in, the river water turns orchid-green with life".

The three most important speeches at the conference were made by Chair-

man Hua, Premier Têng Hsiao-P'ing and Vice-Premier Fang Yi, the latter especially responsible for the development of science and technology in the coming years. The first two were mainly concerned with general principles and the national position of science, while the third dealt with planning in greater detail. Other Vice-Presidents of Academia Sinica, such as the nuclear physicist, Ch'ien San-Ch'iang, also made important contributions.

On 24 March, Chairman Hua Kuo-Fêng said that the country had basically eliminated the chaos created by G₄, and was moving towards great order throughout the length and breadth of the land. The "Four Modernisations" (agriculture, industry, national defence, and science and technology) had been radically subverted by them. Their fundamental heresy had been to stress only one of the three great forms of struggle which Chairman Mao, in his vigorous days, had defined — the class struggle, the struggle for production, and the struggle to understand and control Nature. They confused the people by spreading the idea that class struggle (i.e. moral and sociological orthodoxy) covered everything. Their obscurantism consisted in ignoring the second two struggles which Mao had defined — "it doesn't matter", they said, "if production goes down, so long as our revolutionary attitudes are right"; and as for the scientists and technologists, G₄ despised them and wrote them off as *ipso facto* reactionaries.

Chairman Hua in his speech underlined with much intensity the need to raise the scientific and cultural level of the entire Chinese nation. We need,

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Plenary session of the National Science Policy Conference held in Peking last March.

he said, thousands upon thousands of skilled workers and peasant-farmers who have both socialist consciousness and the ability to master modern production techniques. We need, he said, enormous numbers of revolutionary intellectuals in different trades and professions; and revolutionary cadres capable of managing modern economics and modern science and technology. Hundreds of millions of people, not just a few thousands, must reach a higher technical and scientific level.

Chairman Hua also paid much attention to scientific education. Young people attracted to science should be given every encouragement at all levels. At the same time, the dissemination of popular science for everyone should be encouraged. "Politics", said Chairman Hua "is commander, the soul in everything, and it won't do not to grasp political and ideological work. But neither will it do if we concern ourselves solely with politics and remain laymen without any knowledge of technical and professional scientific activity".

All xenophobia should be done away with. The Chinese should learn the strong points of all nations and countries, and adopt from them all that is truly good in politics, economics, military affairs, science, technology, literature and art. "While upholding independence and self-reliance", he said "we should learn from other countries analytically and critically. We have always opposed the slavish comprador philosophy that holds that everything foreign is good, while nothing Chinese is any good at all, fancying that 'even the moon looks better over foreign lands', and that China can only move along at a snail's pace behind other

countries . . . But if we indiscriminately refuse to learn from foreign countries (as G₄ insisted) China will remain backward for ever". Chairman Hua paid a tribute to some of the greatest Chinese scientists of recent times, such as the geologist Li Ssu-Kuang and the meteorologist Chu K'o-Chen. "Facts past and present", he said "show that we Chinese too have a head and two hands, and are no stupider than other people". Study and again study, unite and again unite, was Chairman Hua's final exhortation at the conference.

A second important speech was by Premier T'eng on 18 March. Dealing with the basic philosophy of science and society, he said the Marxist tradition had consistently held that science and technology are part of the productive forces. It had also held that all scientists, engineers and technicians joined in the creation of surplus value. Accordingly, whether they knew it consciously or not, they also were, for the most part, exploited under capitalism. The only difference between them and manual workers, whether unskilled labourers or trained technicians of the highest quality, lay in a different rôle in the social division of labour. "Those who labour, whether by hand or by brain, are all working people in a socialist society". Yet G₄ had had the effrontery to label all those who worked hard to improve their knowledge and skill, all those who believed that to conquer human ills and discomforts we need to know far more about the processes of nature, as "white reactionaries" or "capitalist-roaders". They made the fundamental mistake of regarding the division of labour between mental and manual work in socialist

society as a form of class antagonism. This led to persecution of the intellectuals, an undermining of their solidarity with workers and farmers, and a deep disruption of the social productive forces, sabotaging the revolution and construction of a new society.

Such points of view are now entirely repudiated in China. "Of course" said Premier T'eng "to raise China's scientific and technological level we must rely on our own efforts, develop our own inventions, and adhere to the policies of independence and self-reliance. But that does not mean shutting the door on the rest of the world, nor does self-reliance mean blind opposition to everything foreign. Science and technology are a kind of wealth created in common by all mankind. Any nation or country must learn from the strong points of other nations and countries, from their advanced science and technology. We shall always have to learn from the strong points of others . . . We must therefore actively develop international academic exchanges and step up all friendly contacts with scientific circles in other countries".

Premier T'eng then turned to his second subject, the building of a mammoth force of scientific and technical personnel who will be both "red and expert". In a socialist society everyone has to remould themselves, and broadly speaking the scientific community was getting on fairly well with this, but under G₄, scientists were greatly distracted by unnecessary political discussion. The conference agreed with Premier T'eng that at least five-sixths of the working-time of scientists should be left free for their proper research and teaching. He went on to emphasise the importance of education in the

training of new scientific and technical personnel; everyone should support it. On the question of young people with special talent, there was a great need to break with convention in discovering, selecting and training them. The history of science, said Premier Têng, shows what great results have been produced in many fields by the discovery and encouragement of young people with genius.

The third question was, how to divide responsible leadership in scientific and technical institutes between the Party committees and the scientific directorates. Here the key for the future would lie in the simultaneous development of the three great revolutionary movements defined by Chairman Mao: class struggle, production struggle, and the struggle to understand and control nature. For this third aspect, the professional scientists and technologists formed the mainstay of the nation. In order to follow out the plans and push forward scientific research, it would be necessary to guarantee the supporting services and supplies, and provide the necessary working conditions for scientific and technical personnel; that too was part of the work of the Party committees. "I am willing", said Premier Têng, "to be the Director of the Logistics Department at your service, and to do this work well, together with the leading comrades of Party committees at various levels." All this means a much freer hand for the directorate of scientific institutions in planning the work in their fields. Premier Têng ended: "May science in China flourish and grow! I wish the conference complete success!"

The third important speech at the conference was given by Fang Yi, one of the Vice-Premiers, a Vice-President of Academia Sinica, and Minister in charge of the State Scientific and Technological Commission. Later we had the pleasure of being received by him for an interesting conversation. His speech presented the "outline National Plan for the Development of Science and Technology". His report proclaimed a new stage in the development of China's socialist science and technology, emphasised the need to foster lofty ideals and set high targets in the march towards modernisation, and called for mobilisation of the whole Party to develop science energetically.

The first plan adopted was an eight-year one, running until the end of 1985, with the aim of high-speed development. Fang Yi said that compared with the rest of the world, China is now lagging some 15 to 20 years behind in many branches, and even more in others. Nevertheless they had fulfilled five years ahead of schedule the

major targets specified in the 12-year plan for the development of science and technology which was mapped out in 1956. In the mid-sixties, China had approached advanced world levels in certain scientific and technical spheres.

The new draft outline plans set out the goals to be attained in the next eight years:

- approaching or reaching the advanced world levels of the seventies in a number of important branches of science and technology;
- increasing the number of professional scientific research personnel to 800,000, if possible, one million;
- building a number of new, up-to-date centres for scientific research;
- completing a nation-wide system of inter-linked, inter-communicating research activities. The eight-year outline plan laid down all-round dispositions for the tasks of research in 27 spheres, including natural resources, agriculture, industry, military technology, transport and communications, oceanography, protection of the environment, medicine, finance and trade, culture and education. One hundred and eight items were chosen as key projects in the nation-wide endeavour.

"When this plan is fulfilled", Vice-Premier Fang said, "our country will approach or reach the advanced world-levels of the seventies in a number of important branches of science and technology, thus narrowing the gap to about ten years, and laying a solid foundation for catching up with, or surpassing, advanced world levels in all branches in the following 15 years" of the 23 before the century's end.

The eight-year outline plan gives prominence to eight scientific and technical spheres. These are as follows:

Agriculture, which itself has an eight-point charter: soil science, fertilisers, water-conservancy, improvement of seeds, study of close-planting, plant protection, field management, improvement of farm tools and machinery. Vice-Premier Fang went into considerable detail in discussing the importance of intensified scientific and technological research in all aspects of agriculture and forestry.

Industrial energy sources. This includes all problems connected with the production of oil, natural gas, coal and other fuels. Among the chief research subjects would be the building of large hydro-electric power-stations, thermal power-stations at pit mouths, large power grids, and super-high-voltage power-transmission lines. At the same time, atomic power generation research should be accelerated, and the building of atomic power plants speeded up. Nor should solar energy, geothermal, controlled thermo-nuclear fusion be neglected. Oil shale and marsh-gas resources should also be looked at.

Materials. Here the focus would be on steel production and also copper and aluminium resources. China is already one of the biggest producers of titanium, vanadium and tungsten in the world, but there is much still to be mastered in modern metallurgical technologies. Cement, plastics and synthetic rubber also came under this head.

Computer technology. At present, China is about ten years behind the rest of the world, but during the coming eight years intends to advance rapidly in all relevant fields, to popularise micro-computers, and put into operation giant ultra-high-speed computers. China will also establish a number of computer networks and data bases. More and more key enterprises will use computers to control the major processes of production and management.

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Chairman Hua: "we should learn from other countries analytically and critically"

Laser optics. In the coming three years, laser physics, laser spectroscopy and non-linear optics will be studied intensively. China expects to make discoveries and new creations during the coming eight years in exploring new types of laser devices, developing new laser wave-lengths and studying new mechanisms of laser generation.

Space science. Although astronautics has not gone far in China as yet, the intention is to build modern centres for space research and the systems for studying, manufacturing and launching a wide variety of satellites.

High energy physics. China expects to build a modern, high-energy physics experimental base in 10 years, completing a proton accelerator with a capacity of up to 50 MeV in the first five years, and one of still larger capacity in the second five years.

Genetic engineering. During the coming three years it is intended to step up the tempo of building and improving laboratories capable of conducting basic studies in genetic

engineering. In due course these will be combined with studies in molecular biology and cell biology. It is planned to use the new technology of genetic engineering in the pharmaceutical industry, and explore feasible new ways of treating and preventing certain difficult and baffling diseases. The aim is also to produce new high-yield crop varieties capable of nitrogen-fixation.

"The march towards the modernisation of science and technology" said Vice-Premier Fang "means in essence a comprehensive and fundamental technical transformation of all fields of material production in our country. This is a great technological revolution which history has entrusted to us". He went on to enumerate the necessity for the following policies:

(1) the consolidation of scientific research institutions, and the building up of a scientific and technological

praising, promoting and rewarding scientific and technical personnel. Here scientists and technologists should be given sabbatical leave to carry out advanced studies, and there should be much more selection of scientific and technical personnel for study abroad in many parts of the world. "We must firmly establish", Vice-Premier Fang went on "the moral code under which it is an honour to produce good results in scientific research for the socialist motherland. Titles should be restored, individual responsibility established for all technical work, and the appraising and promotion of scientific and technical personnel undertaken at regular intervals". Those who have made important contributions to the country should be suitably rewarded.

(4) The policy of "letting a hundred schools of thought contend." Controversy in science and technology should not be discouraged. There should be freedom for criticism and freedom for refuting criticism; and the indiscriminate application of political labels should be strictly prohibited. There should be no bar on scientific publication apart from national security, and scientists who have supported views which have turned out to be in error should not be discriminated against. "We actively advocate", Fang said, "the study of Marxist philosophy by scientific and technical workers, and we ought to encourage and help them to do this. Different kinds of forums should regularly be held, journals on the dialectics of nature (the philosophy of science) should be published, and research on the history of science and technology should be actively encouraged."

(5) The learning of advanced science and technology from other countries, and the increase of international academic exchanges. Scientific and technical co-operation and exchange of personnel with other countries should be greatly strengthened. Scientists and postgraduate students should go abroad for study, and take part in international academic conferences. Foreign scientists, engineering and technical experts will be invited to China to give lectures, to act as advisers or join in scientific research.

(6) Adequate working time for scientists. Scientific research workers should be able to devote at least five-sixths of their working time each week to professional work. The most important projects should be adequately provided with assistants, and the administrative duties of those engaged in important research should be minimised.

(7) Modernisation of laboratory facilities, information and library work. Existing laboratories are to be re-fitted

and modernised, and emergency measures taken to push forward the design and production of instruments and equipment. The distribution and use of scientific instruments under an overall national plan is seen as an essential item to strengthen science in China. The publication of scientific and technological results should be facilitated, and all international literature be made available. Here we may expect also a liberation in the world dissemination of the Chinese scientific journals

(8) Close cooperation with appropriate divisions of labour. Here it is a question of integrating scientific research with production and utilisation. Research institutions of all kinds ought to work in close contact with appropriate factories or hospitals.

(9) Acceleration of the popularisation and application of scientific and technical achievement and new techniques. Here close attention should be paid to the intermediate links between scientific research and industrial and agricultural production. There is great need in China for essential pilot plants and workshops to produce experimentally new products.

(10) Finally the popularisation of science is an aspect of the highest importance. Popular science groups combining the efforts of both professional and lay people should be organised everywhere. Educational science films should be produced on a big scale; museums and exhibition centres should be strengthened, and the press, radio and television should devote more space and time to the dissemination of science and technology. "All sectors", Vice-Premier Fang concluded, "must pool their efforts to foster among the cadres, the masses, and the young people, the habit of loving, studying, and applying science."

So far, no detailed report of the National Science Policy Conference and its conclusions has come to our knowledge, whether in Chinese or a western language, but there can be no doubt whatever of the cardinal importance of the event. As one eminent Shanghai scientist said to us, "The conference was a national mobilisation of scientific man-power to go forward in the most spacious way possible." □

1. Peking Review, 21 (no. 12), 3; (no. 13), 3; 1978; China Reconstructs, 27 (no. 7), 2; 1978.
2. Peking Review, 21 (no. 14), 15.
3. Chung-kuo Hsin-wen, no. 937; 1978.
4. Chung-kuo Hsin-wen, no. 932; 1978.
5. Peking Review, 21 (no. 13), 6; 1978.
6. Peking Review, 21 (no. 20), 6; 1978.
7. cf Peking Review 21 (no. 17), 4; 1978.
8. cf Peking Review 21 (no. 20), 13; 1978.
9. Peking Review 21 (no. 12), 9; 1978.
10. Peking Review 21 (no. 14), 6; 1978.
11. cf Peking Review 21 (no. 12), 29; 1978.
12. cf New China News Agency Bulletin, 1 May 1978, 14.

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Premier T'eng: the need for a "red and expert" scientific workforce

research system. Academia Sinica, he said, must be helped to restore, strengthen, and build new key scientific research institutions. The best universities and colleges must also engage in research and train graduate students. Ministries, provinces, municipalities, and autonomous regions must establish new institutions and strengthen existing ones. In all these laboratories, the system of the scientific directorate assuming responsibility for work with the collaboration of the Party committees must be applied.

(2) The opening of broad avenues to able people, and their recruitment without too much over-emphasis on formal qualifications. Resolute measures, Fang said, must be taken to train gifted people in greater numbers and at a faster rate. Television and radio courses should be developed, correspondence courses, open universities and evening schools. Popular science must be greatly encouraged.

(3) Regulations for training, ap-

Hsinhua

Great leap forward means more contact with West

WHAT conclusions can be drawn from the great events of the past two years which we have been considering in these articles? In the first place, it is clear that the Chinese people are embarking on a programme for the development of science and technology which one would be tempted to call grandiose if China were not about a quarter of all humanity, with the vast stores of talent which this necessarily implies. There has been a steady and immense upsurge of enthusiasm and resolve since the overthrow of the Cabal of Four (G_4) and their followers. As an eminent philosopher of science in Shanghai put it to us, that group has generally been called 'leftist' (*tso p'ai*), but in fact they were more properly speaking highly rightist, because they wanted the people to be ignorant and subservient. To the extent that they were against the high organisation required for advanced science and technology they could be regarded as anarchist in tendency. Other informants analysed it as a kind of fascism, with 'stormtroopers' intimidating the intellectuals and scientists. The cabal as a power-seeking group organised the natural vandalism of the most mean-minded elements in the population. All this has now been done away with, and science and technology have been unshackled in a return to the outlook of Chairman Mao and Premier Chou.

What expectations, then, can we have in the western world? First of all, there will be many new concordats with other national academies, including the Royal Society. As the months go by, there should be much more interchange of people, with more delegations going back and forth, and Chinese scientific personnel at all levels coming to other countries for research, whether graduate students, established scientists, or veteran men of eminence¹. Conversely, there should be a good deal more opportunity for western scientists to go to China, where they will be welcomed, not only for visits and con-

sultations, but, it seems, for joint research projects. This is something definitely new. The limiting factor here will doubtless be the shortage of linguistic man-power, but again this is one of the things which the new line will set out quickly to rectify.

It is quite clear that there has been a changed climate during the present year; for example, the historian of science attached to a recent delegation of American astronomers to China was invited to lecture to the History of Science Institute members of Academia Sinica in Peking. My own experience this spring was similar—in former days one would never be invited to speak, but this time I was asked to give brief lectures and lead discussions in places so far apart as Urumchi and Canton. That there are specialist American engineers employed in Chinese factories we know, and we met two Danish computer experts who had been invited as advisers. We also know of distinguished Chinese-American mathematicians, physicists and embryologists who have spent time in China recently for lecturing and research.

Another forecast concerns the holding of international symposia and even congresses in China. We learnt, to our delight, that the first example of such undertakings will occur later on this year when there will be an international symposium in Peking on plant tissue-culture and the development of haploid plants by anther cultivation. The countries involved in the invitation will be mainly Asian and Australasian, but such a development augurs well for the future. Similarly, the World Health Organisation has been invited to send a strong mission to China early in the New Year to discuss work on the pharmacology of new plant drugs as part of their world survey of these. And a high-level delegation of American government scientists, headed by Dr Frank Press, President Carter's chief scientific adviser, spent a week in Peking in July. They were welcomed by Premier T'eng, and it is expected that a wide range of contacts will follow in the near future.

There is also clearly going to be a return to the earlier years of the revolution when many scientific textbooks and monographs were translated into Chinese from other languages. A translation of the works of Einstein has now appeared, under the aegis of the President of Peking University—something which would have been quite impossible during the Cultural Revolution. Naturally too, we were delighted to see the appearance of some eight volumes of the Peking translation of the "Science and Civilisation in China"

series, commissioned originally by Chou En-Lai himself, but suspended under the régime of G_4 ².

The present year has also seen a great re-organisation of the National Academy (Academia Sinica). At an earlier date, separate National Academies of Medicine and Agriculture were instituted, and these have long been under the control of the respective ministries of Health and Agriculture, but their activities were severely curtailed during the G_4 period. Now a new National Academy of Social Sciences has hived off from Academia Sinica, including the institutes of philosophy and social sciences, archaeology, anthropology, literature, linguistics

6 If they can hold on to the course set by the leaders of the country, there is a chance that by the end of the century China will indeed be among the advanced scientific and industrial nations 9

and philosophy, and dialectical philosophy. There is even an Institute for the study of Religions and Theologies. The history of science, however, is staying with Academia Sinica proper, which of course includes the national institutes of mathematics, physics, chemistry, biological sciences, earth sciences, and many different kinds of technology. In all the social sciences there has been a great liberation of discussion and research³, while in artistic fields there has been a revival of traditional painting, opera and the handicrafts⁴.

At its height the National Academy (Academia Sinica) controlled as many as 200 important scientific institutes throughout the length and breadth of the country, but during the Cultural Revolution and the G_4 period, a great deal of decentralisation took place. For example, the notable Institutes of Botany and the Botanic Gardens at Kunming and Kuangchow (Canton), in Yunnan and Kuangtung respectively, were divorced from central control and placed under the provincial governments in question. Today a compromise is being reached, so that the scientific direction will revert once more to central control, while the administration and funding will be provided locally in such a way as to ensure good horizontal relations with other local and regional institutes. There is also growing up a vast new pattern of organisation which will include not only the Academia Sinica institutes but also the research departments in universi-



Royal Society

Dr M. G. P. Stoker, Foreign Secretary of the Royal Society meets Vice-Premier Fang Yi earlier this month

ties, as well as the laboratories under the control of the industrial ministries, the Ministry of Agriculture and Forestry, or the Ministry of Defence. The specialised scientific societies are also being strengthened and will hold regular meetings for the communication of research results¹.

One of the biggest departures from previous practice is the declared intention to carry out much research in universities, combining it with the training of graduate students. This had been in abeyance for many years because during the twenties Academia Sinica had been modelled on the scientific academies of the eastern European countries, thus concentrating all important research in its own institutions up and down the country. G₄ were particularly against research in universities, and of course not very keen on it anywhere. But now the intake of graduate students will greatly increase. Out of some 400 universities and colleges in China, 100 have been nominated as the most important, and these will be concentrated on first; Peking University itself is one of the most prominent. New institutes will therefore have to be built. The Academia Sinica institutes will also admit postgraduate research students² and will be able henceforward to concentrate more on basic research—not ignoring applications of course—while the other institutes, especially under the various ministries, will do more applied research. In universities both types of work will be emphasised, along with the essential training of graduate students. Much of this was the policy started by Chou En-Lai in 1972.

The question of university entrance constitutes an important crux. During the Cultural Revolution it was agreed that every young man and girl should do one or two years of national service in industry, agriculture or transport before being allowed to take up university work. The first nomination was to come from their fellow-workers, who would take into account the life-style and work-style of the individual, then there would be a certificate from the Party cadres who knew him or her personally; all this in the interests of weeding out 'careerists' and self-seekers' at the start. Then there was an oral examination with the university authorities having the power of veto; for if they considered that the individual did not possess the intellectual capacity needed for university education they could say no. In some cases there were written examinations for entrants, but the powers of the universities were sabotaged by G₄ and their followers, who at one point made into a national hero a young man who handed in blank examination papers. Nothing would make them see that

Hsinhua

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Hu Han (left) of the Institute of Genetics whose research group first developed haploid plantlets of wheat by anther cultivation. China's first international symposium, to be held in Peking later this year, will be on this topic

moral virtue or political orthodoxy will not solve scientific problems or lead to any advanced technology. Actually the young man who handed in blank papers in the examination was, we were told, extremely keen to get in, but he could only answer about 6% of what was asked; he never intended it as a gesture, and then suddenly found himself famous. In any case, written examinations for university entrance have now started again, and the Vice-President of Peking University informed us that this year the scientific level has been remarkably high. Provision is now made for a certain proportion of the intake to be straight from school if the individuals are obviously very bright and brilliant. Normally everyone goes to the countryside, but if they wish it, they can sometimes choose factory production or the transport industry; his own grandson, he said, learnt to drive and repair heavy trucks, but has now gone to a College of Linguistics. In contemporary China there is considerable interest in youthful prodigies, precocious children, and young people of unusual mathematical or other talent.

In the month after the scientific conference, there took place in Peking a National Conference on Educational Work, and Premier T'eng gave an important address to it on 22 April³. As he said, G₄ "were opposed to making any strict demands on students in their study of science and culture, indeed discouraged them from making such work their main pursuit, insisting nonsensically that if they 'put intellectual education first', they would be 'divorced from proletarian politics'." Some pernicious influences of this kind deriving from the cabal were still at large, but they were being steadily eliminated. The full report of the educational conference will also be eagerly awaited.

Premier T'eng went on to say that of course "students must give first place to firm and correct political orientation, but this does not exclude the study

of science and culture. On the contrary, the higher their political consciousness the harder the effort, and the greater the will-power, which the students will exert to acquire scientific and cultural knowledge . . . G₄ were therefore not only absurd in their attitudes, but negated and betrayed proletarian politics when they opposed what they termed 'putting intellectual education first', which was really an effort to improve the quality of education and raise the students' scientific and cultural level . . ." Premier T'eng pointed out that it is not good to put too great a load on students, and here he was evidently criticising the altogether excessive emphasis on political discussion which existed during the reign of G₄. In the same way, while the idea of combining school education with practical productive work was essentially a good one, it was easy to over-emphasise this, as indeed they did. A much more balanced system was necessary. Undoubtedly the Conference on Educational Work, attended as it was by six thousand school-teachers and university lecturers, will exert an important influence on school and university education as part of the overall "grand design" for developing science and technology throughout the country.

All in all, therefore, we are at the present time witnessing great changes in China. If they can hold on to the course now set by the leaders of the country, Chairman Hua and his colleagues, there is a chance that by the end of the century China will indeed be among the advanced scientific and industrial nations. This is no small thing for us to be able to help, and it seems desirable that readers of *Nature* should know what is happening. □

1. cf Peking Review 21 (no. 20), 18; 1978.
2. cf New China News Agency Bulletin, 11 March 1978, 13.
3. cf Peking Review 21 (no. 19), 16; 1978.
4. cf Peking Review 21 (no. 17), 7; 1978.
5. Peking Review 21 (no. 12), 27; 1978.
6. Peking Review 21 (no. 12), 5; 1978.
7. Peking Review 21 (no. 18), 6; 1978.

news and views

It has been known for some time that mice vary considerably in their ability to develop immune responses. In some cases a major part of the variation is apparently controlled by a single Mendelian factor with high responsiveness being dominant. It is now clear that there are at least two fundamentally distinct classes of such genetically controlled immune response defects, each involving a different part of the immune system. One class, expressed in B lymphocytes, is controlled by genes in the chromosome region coding for immunoglobulin heavy chains. It is thought that the responder mice carry a germ line gene coding for the variable (V) region of an antibody which can recognise the antigen in question, whereas non-responders carry a different and inappropriate allele (Blomberg *et al.* *Science* **77**, 178; 1972). The second type of non-response involves T cell functions. Some strains of mice which are known to have B cells capable of making antibodies to particular antigens, do not do so apparently because they lack appropriate T cell help for antibody formation. The Ir genes which govern these T cell deficiencies are linked to the H-2 gene complex (also known to code for the antigens which provoke rapid graft rejection (McDevitt & Benacerraf, *Science* **175**, 273; 1970)). For a long time immunologists have been wondering whether Ir genes operating in T cells are analogous to the immunoglobulin V genes operating in B cells. In other words, do they code directly for the T cell receptor for antigen? The answer seemed to be no, as a different line of experiments had suggested that T cell receptors were encoded by the same set of genes which specified the B cell receptors and were unlinked to Ir. It now seems that Ir genes influence the ontogeny of T cell specificity by controlling the thymic environment in which T cells mature.

H-2 restriction

Why, when the genetics of B cell non-responsiveness seem straightforward, does one ask about the importance of environmental influences on T cell responsiveness? The question springs from evidence that recognition of antigens by T cells is more complex than recognition by B cells. Every T cell

T cell response: nature or nurture

from Polly Matzinger

function involves an interaction with another cell, and this is reflected in the way T cells see antigen. Instead of recognising antigen alone, T cells also take account of the environmental context in which the antigen is presented. Specifically the T cells perceive the animal's own H-2-coded glycoproteins found on the membranes of the cells they encounter. For example, a mouse of H-2 type A recovering from a virus infection will produce T killer cells capable of destroying virus-infected target cells, but only if the targets are infected with the same virus and carry H-2 type A. The 'antigen' recognised by such T cells is thus made of two specific components: virus and self H-2. Similarly, T helper cells normally interact successfully only with B cells of the same H-2 type. So T cell function is said to be 'restricted' by self H-2.

Since H-2 is highly polymorphic, it seemed unlikely that recognition of 'self' H-2 would be a genetically determined property of the newborn T cell. The alternative would be for self H-2 recognition to be set during ontogeny. Recently it was demonstrated that the H-2 type of the thymic environment in which T cells develop does indeed determine which H-2 type they recognise as self (Bevan *Nature* **269**, 417; 1977, Zinkernagel *et al.*, *J. exp. Med.* **147**, 882; 1978; reviewed by Howard *News and Views* **272**, 11; 1978). Heterozygous H-2 type (A×B) T cell precursors, transferred to and allowed to mature in irradiated mice of H-2 type A, are found to be restricted mainly to the recognition of antigen with H-2 type A. Conversely, if homozygous A precursors mature under the influence of an (A×B) thymus, they can under the appropriate conditions produce B restricted T killer cells.

H-2 restriction and Ir genes

What has this to do with Ir genes? An important early clue that was not fully understood at the time came from ex-

periments by Katz and his associates studying the antibody responses controlled by an H-2 linked Ir gene. The (R×NR)_{F1} progeny from a responder (R) by non-responder (NR) mating respond to the antigen. In dissecting the system they found that both R and (R×NR)_{F1} B cells could respond to the antigen if given help from (R×NR)_{F1} T cells; yet NR B cells could not. Non-responsiveness therefore looked like a B cell problem; but a different set of experiments showed that if the NR B cells were given an alternative source of help they responded very well. So the NR B cells could respond, the _{F1}(R×NR) T cells could offer help, but the two did not interact in a fruitful way. H-2 restriction offers a possible explanation for this. Given that T cells seem to recognise antigen in the context of H-2 specified molecules, a particular antigen (X) may be unrecognisable for some reason when presented with the H-2 type of the non-responder mouse. The non-responder would then be incapable of responding to antigen X. T cells from (R×NR)_{F1} would interact successfully with antigen X+R H-2 but could not recognise X+NR H-2 and would therefore be unable to help NR B cells make antibody against X. A description of Ir genes in these terms could explain their linkage to H-2 without invoking direct specification of the T cell receptor by H-2 itself.

H-2 Ir genes don't specify the T cell receptor

So it was known that T cells are H-2 restricted, that their specificity for self H-2 is influenced by the environment in which they mature and that recognition of H-2 is intimately involved with Ir gene controlled responses. Since there was also evidence linking the T cell receptor to B cell V genes, the idea that H-2 linked Ir genes specify the T cell receptor had begun to die a natural death. The *coup de grâce* has now been delivered by von Boehmer, Hass and Jerne (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2439; 1978). They reasoned that, unless Ir genes were actually coding directly for the T cell receptor for antigen, it should be possible to change a NR T cell into a phenotypic R by allowing it to differentiate in an environment where 'self' H-2 is of the responder strain.

The Ir gene they analysed controls T killer cell responses to H-Y, an antigen found on male but not female

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mammalian cells. Female mice can recognise and raise T killer cells against the H-Y on otherwise genetically identical male cells. Such responses are H-2 restricted and controlled by H-2 linked Ir genes. For example, H-2 type i5 (the non-responder in this case) female mice cannot respond to H-Y. H-2 type b (responder) females can, and as expected $(b \times i5)F_1$ females can raise killers specific for H-2 but only when it is presented on cells that also carry H-2 type b. The combination of H-Y plus H-2 type i5 just does not act as a target for T killers. von Boehmer and colleagues reconstituted irradiated $(b \times i5)F_1$ females with non-responder i5 bone marrow (as a source of T cell precursors) and immunised them against H-Y. The genetically NR T cells which had matured in the F_1 environment subsequently behaved like F_1 T cells. They gained the ability to develop T killers against H-Y on H-2 type b cells and remained responsive to H-Y plus H-2 type i5, thus demonstrating that

the appropriate environment can confer responsiveness on a genetically unresponsive T cell population. Conversely, $(R \times NR)F_1$ T cells which had grown up in a NR environment were unable to mount any response to H-Y at all.

This series of experiments tells us that unresponsiveness is not intrinsic to the T killer cell but is a function of its interactions with its environment. We are now left with the question of precisely what the defect is. There are several models proposing ways in which Ir genes could generate T cell recognition deficiencies without directly coding for the receptor itself. One model starts with the assumption that the combination of antigen X plus a particular H-2 mimics a normal mouse membrane component. Consequently, a mouse of this H-2 type would not respond to X because of self tolerance (Schwartz, *Scand. J. Immun.* 7, 1; 1978). Another, proposed by Jerne (*Eur. J. Immun.* 1, 1; 1971) and expanded by von Boehmer

et al. suggests that H-2 gene products 'drive' the diversification of T cell specificity. Thus maturation in the presence of different H-2 types would provide different response patterns to an array of antigens.

It is interesting to note that years ago Tyan and McDevitt (*J. Immun.* 105, 1190; 1970) used radiation chimaeras and thymus transplants in an attempt to answer for T helper cells the question that von Boehmer *et al.* have asked for killer cells, and got confusingly different results. There are several ways of explaining these discrepancies and future experiments will surely resolve the differences. For the time being it seems likely on theoretical grounds that the findings of von Boehmer, Haas, and Jerne will prove to be generalisable. If the original idea was that Ir genes coded for the structure which T cells use to recognise antigen, the modern idea is rather that they specify a part of the antigenic structure which is recognised.

What flattens elliptical galaxies?

from M. G. Edmunds

WHY are elliptical galaxies elliptical? This deceptively simple question may soon be answered. The most obvious explanation would be centrifugal effects. Consider a star, in which the material is held up by thermal pressure against collapse under its self gravitation. If the star is spun slowly around an axis, then it is not hard to understand that it will bulge out at the equator, giving an oblate spheroidal shape. One might extend this argument to galaxies, with the pressure holding the galaxy up against gravitational collapse now supplied by the random motions of the stars. Again, rotational motion about an axis through the centre could give rise to an oblate spheroidal shape. The projected shapes of elliptical galaxies as viewed on the sky are found to have a range of ellipticities, which might be interpreted as due to differences between galaxies in the ratio of kinetic energy in systematic rotational motion and the gravitational potential energy. An estimate of the latter can be obtained from the kinetic energy in the random motion of the stars.

P. Young *et al.* (*Astrophys. J.* 222, 450; 1978) have observed the elliptical NGC 4473, which is quite a flattened galaxy (E5)—on the sky its minor axis is only about half the size of its major axis. Measurement of rotational velocities in elliptical galaxies is very

much more difficult than in spiral galaxies, where individual glowing gas regions with sharp emission line spectra can be isolated for measurement of Doppler shift. In elliptical galaxies there are no such hot gas regions (except sometimes right in the nucleus) and one must rely on the integrated absorption spectrum from the atmospheres of the unresolved stars. The integrated spectrum is broadened by the random velocities of the individual stars, making it hard to distinguish the rotational shifts. By using the techniques of Fourier analysis on spectra obtained at different points across the galaxy, Young *et al.* were able to derive both the variation of rotational velocity with distance from the galaxy's nucleus, and also the intrinsic dispersion in the individual random velocities of the stars. The rotational motion could be followed sufficiently far out that a reasonable estimate of the total rotational energy could be made, and the dispersion in random velocities was found to be constant as far out (3.3 kpc from the centre) as the observations were made. The implied ratio of kinetic energy in the rotational motion about the centre to the kinetic energy in the random motions was found to be much smaller than would be required for the ellipticity of the galaxy to be maintained by rotation as an oblate spheroid. The result reinforces the similar, but less accurate, findings of G. Illingworth (*Astrophys. J. Lett.*

218, L43; 1977) for other nearby ellipticals. In a sample of 13 galaxies he found only 1 with sufficient rotation to be a rotationally-supported oblate spheroid, the other 12 having only a third or less of the required rotational energy.

If rotation is not sufficiently important to flatten the galaxies as oblate spheroids, then why are they flattened? Two possibilities have been proposed, both based on the process of galaxy formation. It is assumed that stars initially condense out from large proto-galactic gas clouds, and the distribution of stars in each cloud subsequently collapses under its own self-gravitation to form a galaxy.

J. Binney has suggested (*Mon. Not. R. astr. Soc.* 177, 19; 1976) on the basis of some simple numerical experiments that, if the initial distribution of stars is highly anisotropic but only rotating slowly, then this anisotropy will persist even though the galaxy collapses to a smaller size. The collapse will eventually be halted by the effects of the random motions of the stars, but Binney proposes that this velocity distribution will itself be anisotropic. Thus the dispersion of velocities along a major axis will be larger than along a minor axis, so that the different 'pressure' of the stars in the different directions will maintain the elliptical shape.

R. H. Miller has also considered the collapse of a system of stars, but his initial conditions are rather different.

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He takes a spherical distribution of stars which is held up by rotation around one axis, and lets it evolve as collapse proceeds parallel to this axis. This extensive calculation (*Astrophys. J.* **223**, 122; 1978) involving more than 100,000 'stars' was carried out using the 64 parallel processors of the ILLIAC IV computer of NASA Ames Research Centre. After following the system for two rotation periods, it had developed into a stable prolate bar (that is, more like a rugby or American football) rotating about one of its shorter axes. This leads to the suggestion that flattened elliptical galaxies are prolate spheroids toppling end-over-end, appearing as ellipses when seen projected on the sky. The apparent rotational velocities of such an object would be lower than those required for an oblate spheroid of similar appearance on the sky, which is at least in the right direction to explain the observations.

In a second paper (*Mon. Not. R. astr. Soc.* **183**, 501; 1978), Binney considers the expected ratio of energy in rotational motion to that in

random motion for both prolate and oblate models, with and without some anisotropy in the velocity distribution. Although he suggests that the observational data obtained so far is at least consistent with all elliptical galaxies being prolate spheroids with isotropic velocity distributions, he prefers oblate spheroids with anisotropic velocity distributions, and there is a hint that the correlation between observed rotational velocity and flattening (admittedly from very sparse data!) is rather greater than might be expected from a distribution of only prolate spheroids.

More observational data are eagerly awaited. With anisotropic velocity distributions, the elliptical galaxies could be even triaxial rather than spheroidal, and observational evidence of this would be provided if rotational motion could be detected oriented along the apparent minor axis of an elliptical. It already seems that we can reject the simple model of an elliptical galaxy as a rotating oblate spheroid with an isotropic distribution of stellar motions. □

More cytochromes

from M. Geisow

Most biochemists are familiar with the red mitochondrial protein, cytochrome *c*. Many may also know that throughout a long evolutionary history since its emergence in photosynthetic and aerobic organisms, cytochrome *c* has been a highly conservative molecule, slow to modify its structure despite the opportunities for change brought by mutation. Emanuel Margoliash and Emil Smith were among the first to note the close homology of cytochrome *c* sequences from diverse species. They also observed that the sequence differences which did occur between each protein reflected rather well the separation of their owners on the evolutionary tree. When the first three-dimensional structures of cytochromes *c* became available, the conservatism of the molecules was at once emphasised and to a degree explained in terms of the biochemical mechanism.

Although some aspects of cytochrome function still remain obscure, the value of the structures of these small proteins to evolutionists is considerable. The latest additions to the cytochrome *c* family were announced recently by R. Dickerson and coworkers (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2674; 1978) and Z. Korszun and F. Salemme (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5244; 1977). Cytochromes *c*₃₅₁ from *P. aeruginosa* (an O₂-respiring organism) and *c*₃₅₅ from *C. thiosulpha-*

tophilum (a green sulphur bacterium) have nearly the same 3-D structure. They are unmistakably related to the previously determined structures of eukaryotic *c*, *c*₃₅₀ (*Paracoccus denitrificans*) and *c*₂ (non-obligate photosynthetic bacteria).

The small size of *c*₃₅₁ originally engendered the proposition that members of this series of cytochromes were evolutionarily distant from eukaryotic *c*, *c*₃₅₀ and *c*₂. However, despite the considerable size differences—*P. aeruginosa* *c*₃₅₁ has 82 amino acids, *Paracoccus* *c*₃₅₀ has 134—the internal and surface regions of these molecules

are closely similar. The haem environments of prokaryotic *c*₃₅₁ or *c*₃₅₅ and the eukaryotic *c* are almost identical. (Fig. 1).

The substantial loss of polypeptide chain in *c*₃₅₁ and *c*₃₅₅ occurs in the region of the propionic acid side chain substituents of the haem group (bottom of molecules in Fig. 1). However, by the introduction of a short helical segment, the small *c*₃₅₁ closes off this region of the molecule. The remainder of the molecule, particularly around the haem group, closely resembles eukaryotic *c*. The structures of cytochromes *c* are yet further illustrations of the remarkable nature of the information carried by the amino acid sequence of proteins. Despite considerable alterations in the type and the number of residues, the overall folding of the cytochrome molecules remains essentially the same.

Unlike many other globular proteins the cytochromes carry definite zones of surface charge. These are, like the haem cavity, also subject to evolutionary constraint. For example, a ring of positive charges encircles the haem face (in the plane of the diagram in Fig. 1). Several lines of evidence indicate that these charged areas represent sites of interaction with cytochrome oxidase. Whatever the precise surface features recognised, they are well enough conserved in *c* for the oxidase from any species to interact with the cytochrome of any other. As expected, this asymmetric charge distribution is also present in the *P. aeruginosa* *c*₃₅₁ structure.

An interesting example of amino acid replacement occurs in the *c*₃₅₅ structure. In *c*, *c*₃₅₀ and *c*₂ a sequentially equivalent (and species invariant) tryptophan holds one propionic acid group of the haem by a hydrogen bond. In *c*₃₅₁ the loss of chain in this region removes the tryptophan. However, an unrelated tryptophan on another chain now assumes this role. Dickerson suggests that this adaptation may be an example of evolutionary convergence.

The structures of *c*₃₅₅ and *c*₃₅₁ place

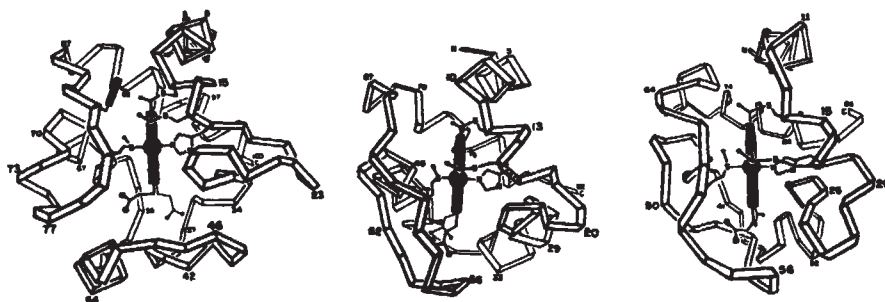


Fig. 1 Mitochondrial cytochrome *c*, *P. aeruginosa* cytochrome *c*₃₅₁ and *C. thiosulphatophilum* cytochrome *c*₃₅₅ (from left to right) (re-drawn from Korszun and Salemme).

them in the same evolutionary group as c_1 , c_{350} and c_2 , indicating a common origin for electron transport chains in photosynthesis and respiration. In fact, c_2 now has species representatives as small as c_{551} . The need for such small polypeptides to carry determinants for a large haem moiety as well as for interactions with oxidase and reductase complexes almost certainly provides an explanation of the very slow evolution of cytochromes. The availability of 3-D-structures makes the task of sequence alignments very much more precise, particularly when coping with deletions of the magnitude found in the cytochrome series. These new cytochrome structures should now allow improved alignments, estimations of sequence differences and hence better measurement of the evolutionary order and separation of photosynthetic and O_2 -respiring organisms. They will also undoubtedly provide more clues to the as yet uncharted interactions with the adjacent proteins in the electron transport chain. □

Neurotoxins in Venice

from Peter Strong and Angela Vincent

THE subtitle of this meeting* speaks to the great impact that neurotoxins have made in the past few years on our understanding of neurobiological events in molecular terms.

The most dramatic advances have been made through the use of post-synaptic α -neurotoxins for characterising the nicotinic acetylcholine receptor (AChR) but controversy continues (see *Nature* 270, 12; 1977) as to whether the acetylcholine 'receptor', as assayed by α -bungarotoxin (α -BuTx) binding, consists of one subunit (J. P. Changeux, Institute Pasteur) or several (A. Karlin, Columbia University, New York; M. Raftery, California Institute of Technology; J. Lindstrom, Salk Institute). The subunit common to all groups (about 40,000 daltons) contains the acetylcholine/ α -BuTx binding site; the other subunits have not yet been ascribed a function. Although the AChR-associated ion channel (as assayed by histrionicotoxin (HTX) binding) is now thought to be a separate entity, the HTX binding protein aggregates in non-ionic detergents (Changeux) and quantitative recovery of the binding activity using cholate extraction has not yet been achieved

(M. Eldefrawi, University of Maryland).

Clues to the function of those putative subunits which are not involved in ACh recognition may come from the use of anti-AChR antibodies. Antibodies raised against *Torpedo* AChR subunits (purified on SDS gel-electrophoresis) cross react with the intact AChR in solution or in the membrane (Lindstrom). Immunised rats develop a mild paralysis, but analysis of the physiological effects of these anti-subunit antibodies *in vitro* may be difficult since anti-AChR antibodies increase the rate at which membrane bound AChRs are degraded (see *Nature* 274, 65, 68; 1978). This also occurs with myasthenia gravis (MG) sera (in which anti-human AChR antibodies are present) and is not accompanied by any change in the rate of AChR synthesis (D. Drachman, Johns Hopkins, Baltimore).

Spontaneously occurring anti-AChR antibodies could also be used as probes to detect differences between α -BuTx binding proteins in crude extracts of tissue. A few patients with IgA deficiency and familial epilepsy have antibodies which bind to an α -BuTx binding protein extracted from rat brain (A. Fontana, University Hospital, Zurich) and which show low specificity towards muscle AChR. MG sera, in contrast, had high anti-muscle AChR titres and very low anti-brain titres. An antigenic difference between limb muscle AChR and ocular muscle AChR was also shown (A. Vincent, Royal Free Hospital, London) although there was no clear relationship between antibodies binding preferentially to the ocular AChR and clinical involvement of eye muscles.

The use of α -neurotoxins to investigate the distribution and pharmacological specificity of AChRs in the central nervous system (CNS) was critically reviewed (D. A. Brown, School of Pharmacy, London). Although a well-recognised distinction between binding of post-synaptic toxins and block of physiological activity now exists at the level of the ganglion, the same cannot be said for the CNS (although Brown pointed out that CNS nicotinic receptors resemble those of ganglia more closely than those of muscle). For instance, peroxidase-labelled α -BuTx bound to synapses in the chick optic tectum (E. A. Barnard, Imperial College, London) and the presence of α -BuTx sensitive activity has recently been shown in toad retinal tectum (*Nature* 269, 218; 1977). Illustrative of the problems in correlating binding with physiological function was the observation that α -BuTx binding to CNS membrane homogenates is dependent on the presence of pre-synaptic dopaminergic

terminals (P. L. McGeer, University of British Columbia, Vancouver). Administration of 6-hydroxydopamine, which destroys these terminals, resulted in loss of binding whereas kainic acid, which destroys glutamate pathways, had no effect. These results could be interpreted as indicating the presence of ACh receptors on dendro-axonal synapses on the dopaminergic axons.

The spectacular successes with post-synaptic neurotoxins have encouraged many laboratories to use pre-synaptic neurotoxins to characterise components of the nerve terminal involved in the regulation and release of neurotransmitters. Results are difficult to interpret, however, because both pre-synaptic snake venom and spider venom toxins have activities which perturb membrane structure—the snake venom toxins have phospholipase A activity and the spider venom toxins are ionophores—and it is not yet clear whether the physiological specificity of pre-synaptic toxins can be related to specific binding to unique components of the nerve terminal membrane. A primary binding step distinguishable and separable from the subsequent steps inhibiting transmitter release could be observed with botulinum toxin (L. Simpson, Columbia University, New York) and phospholipase-inactivated β -bungarotoxin (R. Kelly, University of California, San Francisco) at the neuromuscular junction. Nevertheless, binding evidence is at present indirect and although α -latrotoxin (purified from Black Widow Spider venom) was shown to bind specifically and saturably to synaptosomal membranes from rat cerebral cortex, this toxin did not discriminate convincingly between endplate and non-endplate regions of muscle (M.-C. Tzeng, Rockefeller University). We are still a long way from being able to use these toxins as biochemical probes for pre-synaptic function.

Although the mechanisms underlying generation of action potentials are just as complex as those involved in release of neurotransmitters, nature has provided several different classes of toxin that physiologically delineate the components involved. Tetrodotoxin and saxitoxin plug the sodium channel and bind to an ion-selective filter; batrachotoxin, veratridine and grayanotoxin affect the gating mechanism by maintaining the channel in an open configuration; scorpion toxins and sea anemone toxins also affect gating currents but bind in a membrane-potential-dependent fashion slowing down channel closing. One novel develop-

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* Neurotoxins—Tools in Neurobiology, held at the Fondazione Cini, Venice, 10–14 July, was organised by the Institute of Pharmacology and CNR Centre of Cytopharmacology, University of Milan. To be published by Raven Press.

ment in this area was the report of the synthesis of a photo-affinity labelled radioactive derivative of tetrodotoxin (M. Lazdunski, Université de Nice). The derivative binds specifically and irreversibly to sodium channels after pulse irradiation. This work might open the way to a biochemical characterisation of the sodium channels involved in propagation of action potentials (see also *Proc. natn. Acad. Sci. U.S.A.* 75, 2606; 1978). □

Genetics of industrial microorganisms

from Vedpal S. Malik

At a recent international conference on the genetics of industrial microorganisms*, both the novel techniques of introducing recombinant DNA into industrially important microorganisms as well as more conventional ways of manipulating their physiology were discussed.

Bacterial, fungal and yeast protoplasts are potentially valuable in several ways. One of their uses is to overcome mating barriers between microbes by protoplast fusion and regeneration of a hybrid organism. This technique can be used to breed industrial strains with improved fermentation products, yields or with entirely new metabolites. In fact, P. Hamlyn and C. Ball (Glaxo Laboratories Ltd., Ulverston; UK) have bred a strain of *Cephalosporium acremonium* with a 40% improvement in cephalosporin C titre from the fusion of protoplasts from divergent strains of *Cephalosporium acremonium*. This strain also grows and sporulates better than its highest titre ancestor.

The ability to transform yeast (A. Hinnen, Cornell University Ithaca, New York) and *Streptomyces coelicolor* (D. Hopwood, John Innes Institute, Norwich) protoplasts with plasmid DNA paves the way for the introduction of foreign genes and transposons into industrially important organisms. In this manner industrial strains can be tailor-made by introducing genes of interest and new ways of mutagenesis with insertion elements can be explored.

C. Stuttard (Dalhousie University, observed that reversible loss of chloramphenicol resistance is frequently accompanied by deletions of the nearby arginine locus in *Streptomyces coelicolor*. This suggests

Climatic collaboration

from John Imbrie

UNLIKE physics, chemistry, and mathematics, the science of climatology depends on the availability of a world-wide observation grid capable of recording variations of the global climate system in time and space. Over time scales of months, years, and even decades, climatologists can make use of international data-exchange systems such as the World Weather Watch. But to date, the exchange of information on the lower frequencies of climatic variance — of the sort studied by palaeoclimatologists — has occurred almost exclusively through the normal, spontaneous process of scientific research and publication.

One exception to this rule is the system of International Commissions set up by the INQUA Congress, especially the Commission on Palaeogeographic Atlas of the Quaternary, now chaired by A. A. Velichko of the Institute of Geography of the Soviet Academy of Sciences. Another exception is Working Group VIII of the US-USSR Bilateral Agreement on the Environment. Set up in 1972 as one of the fruits of the Nixon-Kissinger diplomacy, the members of Working Group VIII try by various means to promote the exchange of scientists and data bearing on the problem of climate and climatic change.

Under the auspices of Working Group VIII, six US palaeoclimatologists visited Moscow and Baku during November, 1976, and participated in the First US-USSR Conference on Palaeoclimatology. From 18-27, June this year the same US delegation (John Imbrie, Herbert Wright, Ted C. Moore, James D. Hays, Thompson Webb and Stephen Porter) served as hosts to a Soviet delegation (Academician I. P. Gerasimov, Ye. Gurtovaya, A. A. Velichko, V. Khodakov, and F. V. Mamedov).

One outcome of the visit, which included a scientific conference and field trips to classic Quaternary exposures in the US was the signing of a communiqué in which specific plans were laid out for publishing a National Monographs series, with volumes appearing in both English and Russian. The Soviet monograph would describe the last glaciation of the USSR, including its degradation and the development of the modern landscape. The US monograph would appear in two volumes, the first dealing with the interval 25,000–10,000 BP, and the second with the past 10,000 years.

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that chloramphenicol resistance in *Streptomyces* could be located on an insertion-type element.

C. Stuttard (Dalhousie University, Halifax) reported the isolation of virulent transducing phage in a chloramphenicol-producing streptomycete. There were many other reports on plasmids and phages of streptomycetes but their role in antibiotic formation has not been conclusively established. Genetic studies suggest that methylenomycin in *S. coelicolor* and chloramphenicol biosynthesis in *S. venezuelae* is plasmid-controlled but plasmid DNA that would lend support to this view has not been isolated (H. Schrempf, Institute for Genetics and Microbiology, Wurzburg; L. C. Vining, Dalhousie University, Halifax). There is at present no known streptomycete plasmid that has a well characterised biochemical marker and can be physically isolated. Such a plasmid could help in developing a cloning system in streptomycetes.

New derivatives of anthracycline glycosides have been obtained by cosynthesis with blocked mutants of

Streptomyces griseus and *S. violaceus*. A new anthracycline antibiotic iremycin (a derivative of violamycin) has been produced by an interspecific recombinant. This recombinant, called *S. violaceus* ss iremycius was obtained after hybridisation between blocked mutants of turimycin (a macrolide antibiotic)-producing *S. hygroscopicus* and violamycin (an anthracycline)-producing *S. violaceus* (W. Fleck, Central Institute for Microbiology and Experimental Therapy, Jena).

E. Cerda-Olmedo (University of Seville) reported that unstable heterokaryons of phycocyanins make increased amounts of β -carotene. Such heterokaryons were stabilised by introducing a system of balanced lethal mutations and may therefore have industrial applications.

Mutants of *Nocardia mediterranei*, one blocked in the transketolase and another in shikimate kinase, accumulate D-ribulose and shikimate respectively in amounts equivalent to

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*The Third International Symposium on Genetics of Industrial Microorganisms was held on 4-9 June, 1978, at The University of Wisconsin in Madison. The proceedings will be published by the American Society of Microbiology.

the rifamycin B produced by the parent strain. This suggests that primary metabolites (like ribulose) are channelled in equimolar amounts to synthesis of secondary metabolites. By manipulating regulation of primary pathways one could therefore increase yields of antibiotic and other fermentation products (Neusch; Ciba-Geigy Ltd., Basle).

Penicillin is synthesised by condensation of L- α -amino adipic acid, cysteine and valine to form the tripeptide δ -(L- α -amino adipyl)-L-cysteinyl-D-valine. Activated cystathione and not cysteine may be the immediate precursor of sulphur in the formation of β -lactam antibiotics (Neusch). This tripeptide may give rise to the β -lactam nucleus. The α -amino adipic acid is the precursor in penicillin biosynthesis as lysine auxotrophs of *P. chrysogenum* blocked after the synthesis of α -amino adipic acid produce penicillin whereas those blocked before the synthesis of α -amino adipic acid do not. In *Penicillium* exogenous lysine inhibits penicillin production by curtailing the intracellular supply of α -amino adipic acid. P. Masurekar and A. Demain (Massachusetts Institute of Technology) suggested *in vivo* inhibition of the homocitrate synthetase by lysine. In lysine auxotrophs of *P. chrysogenum* homocitrate excretion is completely inhibited—if the culture is supplemented daily with 50 mM lysine. Homocitrate synthetase activities of cell-free extracts of the parental *Penicillium chrysogenum* grown in complex medium supplemented with 50 mM lysine at inoculation time, or with daily 50 mM lysine additions, were respectively 10% and 42% less than control cultures. Inhibition is complete, however, when *P. chrysogenum* is grown in a synthetic Czapek-asparagine medium in which no penicillin synthesis takes place. The sensitivity of homocitrate synthetase to lysine inhibition in cell-free extracts of the high penicillin producer is identical (J. Martin, University of Salamanca).

Z. Hostalek (Czechoslovak Academy of Sciences, Prague) presented evidence that malonyl CoA for the biogenesis of chlorotetracycline in *S. aureofaciens* is not derived from acetate but from oxaloacetate. Acetyl-CoA carboxylase is not present in high chlorotetracycline producing strains during antibiotic production. The low activity of acetyl CoA carboxylase in *S. aureofaciens* during chlorotetracycline production is correlated with low activity of pyruvate kinase and pyruvate dehydrogenase complex. This indicates that the conversion of phosphoenolpyruvate to acetyl CoA is suppressed when chlorotetracycline is being produced. Phosphoenolpyruvate carboxylase is

very active yielding oxaloacetate which is further channelled towards malonyl CoA synthesis, a precursor required for chlorotetracycline biogenesis.

The growing interest in using microbes for the commercial production of fine chemicals, therapeutic agents, enzymes, insecticides and other products for human consumption indicates the importance of this symposium. In the United States about 200 corporations and world-wide more than 500 companies are utilising microbes to make metabolites useful to man. Recent advances in the genetics and regulatory biology of industrial microorganisms prove that microbial processes can indeed be made more efficient and economical by genetic manipulation of microbial strains in use at present. □

Estimating human mutation rates from polymorphisms

from J. H. Edwards

E. B. FORD defined polymorphism as "the occurrence together in the same locality of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation." At that time loci were generally believed to be few and allelic variation limited. The electrophoretic resolution of proteins later demonstrated a wealth of variation and the extent of this variation was almost unlimited, since most proteins had a hundred or more amino acids, each of which could change in one step in a dozen or so ways, and in two or three steps in some twenty ways.

The possible variants of any one protein were therefore more numerous than the total ancestral population of any mammalian species and the concept of recurrent mutation, and of mutation pressure, was no longer plausible as a cause of specific protein variation. However, even if recurrence is removed from Ford's definition, the problem of how rare is rare remains. Given a mutation rate μ from the wild type, and a population of size N of whom n are sampled, what is the chance of an allelic variant of no selective influence (a neutral allele) persisting by chance to exceed a number m after t generations.

How rare is rare? Thompson and

Neel (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1442; 1978) using a lucid and laconic numerical argument have derived the probability distribution and vindicate their derivation by simulation. They (Neel & Thompson, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1904; 1978) then pose the question of what is the value of μ which is most likely to lead to the proportions of those alleles known only in some of the twelve tribes of American Indians from South and Central America which Neel and his colleagues have studied. That is, given N, n, m and t what is μ . Then, assuming N varies in a plausible way, estimate μ again.

To derive this they need to estimate the number of ancestors connecting the present generation to the founder allele, and assume that mutation was equally likely to occur in any of these ancestors. How many ancestors has a set of $2n$ alleles dispersed over a total population of N individuals? In a population consisting of families of two children, for example, each allele would have a 1/4 chance of being passed to neither child, giving a 0.75 adult-to-adult allelic transfer. If, for example half the population had no children and the other half had four, all of whom survived, the transfer would be about half (7/16). If it were half, the ancestral numbers would be 1,2,4,8... and their total one less than the present population. In practice, matters are less tidy, but it is usually a little less than a half. That is, a closed population of $2N$ alleles has of the order as many ancestral alleles reaching back to the founder allele. A sample must have fewer, but their estimation is more difficult.

By analysing data from part of a closed community of known size Neel and Thompson have been able to make an estimate of the mutation rate, at the level of the primary gene product, without having to test a number of individuals exceeding the reciprocal of the mutation rate, and without confusion from uncertain paternity. Their estimate, of between 10^{-5} and 10^{-6} for mutants evident on electrophoresis, provides the only substantial data yet available in any mammal for the mutation rate from a defined genetic unit smaller than a chromosome. Most studies in man are not related to defined units, since the nature of most dominant disorders is unknown, while the pigmentary and other variants used in mice are not yet understood in molecular terms.

The authors necessarily assume that mutation is independent of locus, place, time and race. However, the finding of two albumin variants is itself surprising. If we extend the family of man more widely the uniformity of albumin seems inconsistent with the

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mutation rates found. The authors have explained why some Amerindians differ from each other, but their explanation only deepens the mystery of why the brotherhood of man is so uniform at some loci.

For the first time we now have a plausible estimate, with confidence limits, of the mutation rate for neutral allelic variants. Harmful allelic variants, or complete allelic deficiencies, may or may not mutate faster, but at least the estimate relates to the unit responsible for recessive disorders in man. These are the major genetic burden in practice, even if dwarfed by the incidence of other genetic disorders in those naturally aborted, and exceeded by that of dominant disorders in the retired. □

What is a myosin? : *Acanthamoeba* revisited

from Paul Wagner

ONE of the more fascinating aspects of the contractile proteins from *Acanthamoeba castellanii*, a soil amoeba, is its rather exotic 'mini' myosin (Pollard & Korn, *J. biol. Chem.* **248**, 4682, 4691; 1973). All other non-muscle myosins so far isolated are structurally similar to the well characterised muscle myosins (Korn *Proc. natn. Acad. Sci. U.S.A.* **75**, 588; 1978; Clarke & Spudich *A. Rev. Biochem.* **46**, 797; 1977). They have monomer molecular weights in the region of 500,000 and are thought to be composed of two heavy chains of molecular weight around 200,000 and two pairs of light chains of molecular weight about 20,000. Muscle myosin has a rod-like tail which is responsible for aggregation into thick filaments and two globular heads which bind to actin and hydrolyse ATP. The one exception has always been the small myosin from *Acanthamoeba*. Its molecular weight is only 180,000 being composed of three subunits with molecular weights of 140,000, 16,000 and 14,000. Although it is believed to be globular in nature, its physical properties are as yet inadequately defined, for instance, whether it forms dimers. Its ATPase activities conform to those of muscle myosin. The low Mg^{2+} ATPase is stimulated 20-fold by actin but only in the presence of a cofactor which has recently been shown to be a heavy chain kinase

(Maruta & Korn *J. biol. Chem.* **252**, 8329; 1977). The myosin binds to actin in the absence of ATP but does not show the marked polarity or 'arrow heads' observed with other myosins. Mg^{2+} ATP dissociates the actomyosin complex. This mini-myosin does not form thick filaments *in vitro* which may indicate a contractile system somewhat different from that of skeletal muscle.

The criteria used by Pollard and Korn in the isolation of this small myosin, now referred to as *Acanthamoeba* myosin I, were a high ATPase activity in K^+ EDTA and a Mg^{2+} ATPase which could be stimulated by actin. However, subsequent experiments with *Acanthamoeba* cell extracts have led to the isolation of a second myosin. Pollard (*J. Cell Biol.* **68**, 579; 1976) found that when cell extracts were warmed in the presence of Mg^{2+} ATP they formed a gel which then contracted or, more precisely, underwent syneresis, expulsion of interstitial fluid. SDS gel electrophoresis of the contracted gel showed a band of around 160,000 molecular weight in addition to actin and several other components. Pollard assumed this to be the heavy chain of *Acanthamoeba* myosin I. However, he was unable to reconstitute a gel with this myosin and its cofactor which would undergo syneresis. Maruta & Korn (*J. Cell Biol.* **252**, 399; 1977) realised that the band observed on SDS gels was too large to be the heavy chain of *Acanthamoeba* myosin I. Moreover, they found that they could not cause a reconstituted gel to contract upon the addition of myosin I and its cofactor, but contraction was observed if a second type of myosin, *Acanthamoeba* myosin II, was added.

Acanthamoeba myosin II has been subsequently purified and characterised independently by Maruta & Korn (*J. biol. Chem.* **252**, 6501; 1977) and by Pollard *et al.* (*J. biol. Chem.* **253**, 4798; 1978). Structurally it is a more conventional myosin with a molecular weight of 400,000 being composed of two 170,000 dalton heavy chains and three or four light chains. Platinum shadowing has shown it to have two globular heads attached to a 90 nm tail (Pollard *et al. J. biol. Chem. op. cit.* 1978). Myosin II forms bipolar thick filaments similar to those observed for other cytoplasmic myosins and binds to actin in the absence of Mg^{2+} ATP but not in its presence giving the characteristic arrow head structures. It has a low Mg^{2+} ATPase which can be stimulated by actin, albeit only 0.5 to two times. Other myosins also show low levels of stimulation by actin. Smooth muscle and platelet myosins require phosphorylation of one of their light

chains for actin activation (Adelstein *Trends Biochem. Sci.* **3**, 27; 1978) and *Acanthamoeba* myosin I requires phosphorylation of its heavy chain. It is possible that a modification system also exists for *Acanthamoeba* myosin II which would allow for a greater activation by actin.

An obvious area of concern is whether myosin I is a proteolytic fragment of myosin II. The results of Pollard and coworkers indicate that there is a 200,000 molecular weight species in equilibrium with the 400,000 molecular weight myosin II. Loss of a 30,000 molecular weight fragment from the heavy chain could easily shift the equilibrium towards the monomer. However, both groups present evidence that this is not the case. The ATPase activities of the two myosins are very different. Myosin I's heavy chain kinase has no effect on myosin II. Myosin II has 32 cysteine residues per mol while myosin I has none. Increasing the extraction time does not alter the relative amounts of the two myosins and 3H -myosin II added to cold *Acanthamoeba* before homogenation was only found in the myosin II peak. While it is possible to criticise the validity of some of these observations, and one hopes that immunochemical tests and peptide mapping will be performed, the circumstantial evidence strongly indicates that the two myosins are not related by proteolysis.

If as seems likely, myosin I is not a proteolytic fragment of myosin II, this will be the first example of two myosins in a single non-muscle cell. The amoeba, like many other cells, performs a variety of motile activities, such as cell movement, endocytosis, cytokinesis and chromosome movement, all of which may rely on the interaction of actin and myosin. Independent regulation of these activities could be achieved either by using different control systems for the same myosin or by using more than one type of myosin and accompanying control systems. Neither Pollard and his associates nor Maruta and Korn believe that the presence of more than one myosin in *Acanthamoeba* is unique but suggest that other cells on closer examination may show more than one myosin type. Indeed there are hints that there may be an *Acanthamoeba* myosin III.

Most models of cellular motility are based on the sliding filament theory of muscle contraction. Thus cells are viewed as containing 'mini muscles' with interdigitating thick and thin filaments which are connected to the plasma membrane or various intracellular structures. Such a view may prove to be limited and unimaginative. *Acanthamoeba* myosin I challenges

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this conventional view, but we must remember that it has not yet passed the final and most important test for a myosin, which is to show that its actin activated ATPase will cause movement. $\square$

Coupled oscillators in chaotic modes

from N. MacDonald

THE realisation that systems with apparently simple, but non-linear, dynamics can exhibit quasi-random behaviour has provoked much activity in the past few years. The Lorenz hydrodynamical model, the launching pad for much of this work (see *News and Views* 271, 305; 1978) continues to yield rich results. Numerical investigations reveal (Shimizu & Morioka *Phys. Lett.* A66, 182, 447; 1978) that with increasing Rayleigh number one passes between successive regimes of periodic and chaotic behaviour. The topological nature of the attractors in this model has been examined in some detail (Rand *Math. Proc. Camb. Phil. Soc.* 83, 451; 1978). It is becoming clear that a wide variety of non-linear systems display analogous chaotic behaviour. It is therefore natural to seek such behaviour in models of forced or coupled non-linear oscillators, in view of their extensive application in physics, engineering and other disciplines.

It is rather characteristic of the whole subject of non-linear dynamics that the investigation of specific examples is the only way through the abundance of phenomena that emerge once the simple linear law of superposition of solutions is abandoned. There are traditionally two popular models of non-linear oscillators. The Duffing equation

$$\frac{d^2x}{dt^2} + ax + bx^3 = 0$$

originated in corrections to the ideal behaviour of a mass on a spring. The Van der Pol equation

$$\frac{d^2x}{dt^2} - c(1-x^2)\frac{dx}{dt} + x = 0$$

originated as a model of oscillations in a triode circuit. Recently the Brusselator,

$$\frac{dx}{dt} = A - Bx + x^2y,$$

$$\frac{dy}{dt} = Cx - x^2y,$$

has been introduced as a crude model of chemical oscillations. (It is necessarily crude because the cubic term, taken literally, implies a collision between three molecules.)

When any of these oscillators is subjected to a sinusoidal input it shows the phenomena of simple and subharmonic entrainment. When the frequency of the input is fairly close to the natural frequency of the oscillator, or to some low multiple of that frequency, the nonlinear oscillator goes into stable periodic motion. The range implied by 'fairly close' depends on the amplitude of the input. Recent numerical studies of the Brusselator (Tomita & Kai *Phys. Lett.* A66, 91; 1978) show that at input frequency near to twice the natural frequency, for sufficiently large input amplitude, the subharmonic oscillations become unstable and are replaced by recognisably chaotic behaviour. These authors use a 'stroboscopic' method, in which one examines the trajectory of the point (x,y) at successive times separated by the period of the driving force. The continuous dynamics of the model can assimilated to the well-studied dynamics (see the review by May *Nature* 261, 459; 1976) of the onset of chaos for a discrete dynamical system. To borrow a phrase from Oscar Wilde, this is "chaos illuminated by flashes of lightning".

An investigation (Fujisaka & Yamada *Phys. Lett.* A66, 450; 1978) of sets of two or three coupled oscillators similar to the Duffing oscillator has also revealed chaotic behaviour. A practical device for demonstrating such effects in coupled nonlinear oscillators has been described (Gollub, Brunner & Danby *Science* 200, 48; 1978). They use two tunnel diodes, each connected in series to an inductor and a resistor. These units are connected in parallel, through a resistor, to a D.C. source. Oscillations are observed in the voltage across each diode, with frequencies in the ratio of two integers, the particular ratio depending on the source voltage. Next an additional resistor is used to link the two halves of the circuit. It is now possible to find aperiodic variation of the voltages across the diodes, demonstrated as a noisy power spectrum.

These results are more than a mere curiosity, or illustrations of a topic currently fashionable in mathematics. Forced and coupled oscillators have considerable relevance, for example, to a great variety of phenomena in physiology, covering time scales from seconds to weeks. Some examples may be cited here. Electromagnetic signals from the gut (Linkens, Taylor & Duthie *I.E.E.E. Trans. Biomed. Eng.* 23, 101; 1976) display complex spectra that sug-

gest that both the nature of individual oscillators and their mode of coupling require investigation. Contemporary discussion of the origins of circadian rhythms leads one to the investigation of populations of coupled oscillators. (See review by Winfree *Nature* 253, 308; 1975). The frequency spectra of the fluctuations of concentrations of blood cells of various types in the peripheral blood of collie dogs or of human patients with cyclical neutropenia suggest that one may be dealing with coupled oscillating cell lineages (see Date *et al. J. clin. Invest.* 51, 2197; 1972; Guerry *et al. J. clin. Invest.* 53, 3220; 1973 for the data, and Kurland *et al. Science* 199, 552; 1978 for some possible modes of coupling.)

The classification of possible new modes of behaviour of coupled oscillators, and the elucidation of which, if any, of these modes are universal and which depend on the specific nature of the oscillators or of their coupling, is a necessity for the full development of models for such situations. Even when it is clear that the normal functioning of the system is periodic one may wish to model pathological arrhythmic conditions (Mackey & Glass *Science* 197, 287; 1977). The numerical and experimental investigations reported above represent steps towards this goal. $\square$



A hundred years ago

WE learn from *Harper's Weekly* that, for the purpose of prosecuting biological researches, Prof. A. Agassiz has lately completed a superb establishment near his residence at Newport, wherein every device that experience could suggest has been brought to bear for the convenience of investigators. A building 45 by 25 feet has been erected on the side of a bay making up from the entrance to Newport Harbour, and provided with the purest of sea-water by means of a steam-pump, which keeps a tank constantly filled. The tables are covered with a series of tiles of different colours, so that the minute animals of different shades can be the more readily overhauled when emptied upon them. The shelves in the laboratory are all of glass, the tanks are of slate, the conducting pipes are of iron, lined with a composition of rubber, which it is believed will protect them against corrosion. These tables are all well lighted, and are available for students, whom Mr. Agassiz invites to share his facilities.

From *Nature* 18, 29 August, 466; 1878.

Metallic glasses: the reason why

from Robert W. Cahn

METALLIC glasses in great variety have been made by quenching appropriate liquid alloys at ultrafast rates, 10^5 – 10^8 K s^{-1} , into the form of thin ribbons or disks. Most such glasses consist of metal/metalloid combinations but some are also made from metal-metal melts. The degree of (meta)stability of such a glass against crystallisation can be measured in two ways: the critical cooling rate required to inhibit crystallisation during the quench can be estimated, or the temperature at which the glass begins to crystallise on reheating can be determined. The former measure, sometimes called 'glass formability', is to be preferred, because it is related to the maximum rate of crystallisation of the glass (located at a temperature well above that of incipient crystallisation); it is the maximum speed at which a crystal can consume the glass that matters, not the temperature at which it happens.

A question which has exercised metallurgists and physicists ever since the first liquid-quenched metallic glass was discovered in 1959 is: what atomic or structural features determine the degree of glass formability? Three alternative models have been proposed. They are not mutually exclusive, but it is nevertheless of considerable interest to know which factor is the crucial one for any particular glass, or even for metallic glasses in general. One model, formulated by Polk in 1972, envisages simply that metallic atoms form a dense random packed array and the smaller metalloid atoms fill the voids in that array and thereby stabilise it. This model cannot be of universal validity, since it cannot account for the formability of the metal-metal glasses. A second model, originated by Chen and Clark in 1973, points to the X-ray evidence for the existence of short-range chemical order between first-nearest neighbour atom pairs in metallic glasses and melts (evidence which has continued to accumulate) and attributes easy glass formability to the enhancement of cohesion in the liquid/glass resulting from strong, chemical bonding between unlike nearest neighbours. The third model, conceived by Nagel and Tauc in 1975, attributes the high resistance of certain glasses to crystallisation to purely electronic factors. This last model, intended as a critique of the other two, seemed to be riding high, but now its claim to universality has been overthrown by a new experimental study (Bauhofer & Simon *Phys. Rev. Lett.* **40**, 1730; 1978).

The free-electron model of S. R. Nagel and J. Tauc (*Proc. 2nd Int. Conf. Rapidly Quenched Metals* (ed. Grant & Giessen) 337, MIT Press, 1976; *Solid State Comm.* **22**, 129; 1977) is in effect a modification of Mott and Jones' classical model for the stability of certain crystalline phases such as β CuZn. Nagel and Tauc point out that a glass must have a spherical Fermi surface of radius k_F and, although no reciprocal lattice vectors exist, there is an analogous quantity, which is experimentally determinable; this is the structure factor $S(Q)$, a function of reciprocal distance, closely related to the interference function and the pair distribution function. The structure factor of any glass (or liquid) always has a high and narrow first peak, at reciprocal distance $= q_F$, and the main postulate of Nagel and Tauc is that high glass formability is favoured by the condition $2k_F = q_F$. This is equivalent to the condition that the Fermi energy E_F lies at a minimum of the density of states curve: this in turn implies that the glass reposes in a metastable minimum of electronic energy with respect to changes in composition and therefore in E_F . In their 1977 paper they explain, ingeniously, how this criterion can lead naturally to a preference for unlike nearest neighbours without the need to postulate strong interatomic bonds, though the nagging suspicion will not go away that this distinction amounts merely to semantic sleight-of-hand! They also show that a number of actual glasses in the Au-Si, Au-Ge and Co-P systems obey their criterion.

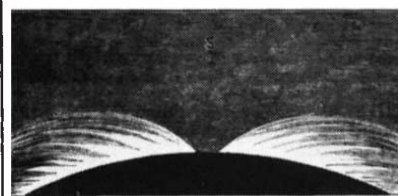
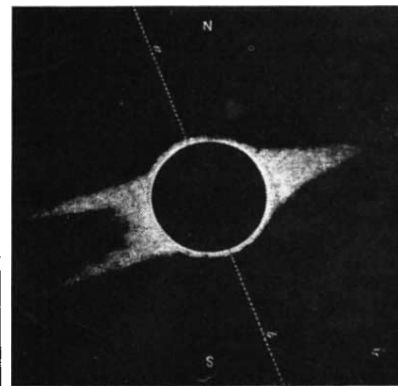
What Bauhofer and Simon have done is to make and examine glasses in two unexpected systems, Cs-O and Rb-O, and show that these glasses do not at all obey the Nagel-Tauc criterion. Though pure alkali metals are stereotypical non glass formers, Cs-O melts can be turned into glasses over the range 15–20 at.% O, and Rb-O over a narrow range near 13 at.% O (which does not incidentally coincide with the eutectic minimum at ~ 9 at.% O); the Rb-O glasses have a small admixture of crystallites. These glasses all crystallise at about 200 K, so that to make them, a sub-zero quenching medium is needed. It turns out that quenching the melt, contained in a 2mm capillary, direct into liquid nitrogen is effective, in spite of the fact that the cooling rate cannot exceed 100 K s^{-1} .

Bauhofer and Simon also measured the X-ray scattering of their glasses and thus located the first peak of $S(Q)$ in each case. They then adduce evi-

dence that oxygen dissolved in liquid caesium removes two electrons per oxygen atom from the conduction band to form an O^{2-} ion; this also happens in crystalline Rb and Cs suboxides. They conclude that the Fermi level can be computed on this basis, and when this is done it turns out that, for both glasses, k_F coincides accurately with the valley between the first two peaks of the structure factor. Thus, $2k_F = q_F$ is not obeyed for these very readily formable glasses, and the authors conclude that strong though non-directional chemical bonding in the glasses is the basis of their stability. To form, for instance, crystalline caesium suboxide, large, strongly bonded $Cs_{11}O_3$ clusters have to form first and this is bound to be slow; presumably similar clusters exist in the glass. The electronic criterion of glass formability, whatever its merits eventually turn out to be, is not universal. $\square$

In July 1878 the editor of *Nature*, Norman Lockyer, watched the eclipse of the Sun from the station of Separation in Wyoming. "... I give a rough sketch of what I saw of the corona with the naked eye (top), slightly exaggerating the dimensions of the streamers to show the wind-vane appearance, which, to me, was almost perfect, being pointed at one end and bounded by parallel lines at the other; others I may say, however saw a resemblance to a fish's tail. These streamers vanished absolutely in the telescope (bottom), as did the radiation lines in 1871; not a shred of them was left.

From *Nature* **18**, 29 August, 457; 1878.



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review article

Selfish genes, evolutionary games, and the adaptiveness of behaviour

G. A. Parker*

The science of sociobiology, which began in principle with the work of Fisher and Haldane and has more recently been developed by Hamilton, Maynard Smith, Trivers, Wilson and others, has been the centre of both scientific and political controversy. Dr Parker discusses the strengths and weaknesses of the approach, and illustrates that behaviour can be adapted in a complex way in conformity with sociobiological theory.

WE all share an interest in why we behave the way we do. In the absence of belief that our nature is determined by supernatural forces, we must seek to explain our behaviour in terms of experience or genetic predisposition; concepts such as free will (unless compatible with physical determinism¹) become largely abstractions.

The study of animal behaviour has benefited from controversies arising out of a series of independent developments. The American 'behaviourist' school pioneered by Watson² and Thorndike³ and the 'reflexology' of Bekhterev⁴ and Pavlov⁵, concentrated exclusively on learning as a behaviour process perhaps largely because they chose to work on higher vertebrates. This resolved into the Skinnerian⁶ view that almost all observable behaviour is the product of experience through reinforcement and conditioning. Behaviour patterns are seen as chain reflexes shaped by the history of reinforcement episodes, and man's behaviour is considered to be the totally plastic product of environmental influence. 'Ethology', on the other hand, originated from the work of early naturalists, especially Darwin⁷, and developed in particular from the works of Lorenz⁸, Tinbergen⁹ and von Frisch¹⁰. Ethologists emphasised innate aspects of behaviour and stressed that its evolution could be derived by the phylogenetic approach in exactly parallel fashion to morphological developments. They usually discussed the adaptive values of the behaviour patterns they described.

A difficulty commonly arose here, because of the commonplace adoption by biologists of an unfortunate shorthand; namely that one could equate darwinian fitness with 'advantage to the species'. This is easy to understand; often a simple advantageous mutation will spread to fixation and at this point there is an overall benefit because the species is the sum of all its individuals. However, the shorthand was particularly regrettable because it obfuscated the vital darwinian principle that in order to spread in the population, a characteristic must convey an advantage to the individual possessing it. Thus ethologists often proposed adaptive values for behaviour that (although conceivably advantageous to the species or social group) were in conflict with individual advantage. In exactly the same way, ecologists often concentrated on population and community levels in their interpretations of function. For far too long, developments in population genetics remained largely ignored by ethologists and ecologists¹¹.

The 'advantage to the species' bubble burst soon after the publication of V. C. Wynne-Edward's¹² famous book on social behaviour in animals. Wynne-Edwards proposed that social

displays had evolved to convey information about population density. Animals were assumed to monitor population density and also the current carrying capacity of the environment. As population density approached the carrying capacity, animals were expected to show 'reproductive restraint'; seen as an adaptative avoidance of disastrous population crashes due to overexploitation of environmental resources¹³. Although of advantage to a local group, this adaptation requires that individuals display biological 'altruism'; that is a mutant with the character (reproductive restraint) sustains a loss in darwinian fitness.

Clearly, if altruism is to evolve, it requires modes of selection rather different from that conceived by Darwin. Wynne-Edwards suggested that group selection (first proposed by Carr-Saunders¹⁴) might be operative. Roughly, he proposed that altruism can evolve if the advantages to survival of a group overrides the negative effects of selection acting on individuals. Whether group selection is plausible became a major controversy in biology¹⁵⁻²⁹; Williams gave a most notable early critique, and so more recently has Dawkins²⁷. (See especially the recent review by Maynard Smith²².) The debate is not entirely resolved but it is generally accepted that group selection will normally be a very weak selective agent by comparison with individual selection, and a solution to be invoked only when certain extreme conditions (small group size, low migration rates, rapid extinction of groups containing any non-altruists) are satisfied²². Biological altruism can evolve through what Dawkins²⁷ would call the 'fundamental law of gene selfishness'. Innate behaviour strategies evolve by the enhanced replication of their genetic determinants, but this underlying selfishness can take the guise of apparent altruism in certain circumstances (see next section).

A significant by-product of the group selection controversy was the catalysis of developments in the common ground between population biology, behaviour and ecology. The fruits of this fertilisation have recently been gathered in masterly fashion by E. O. Wilson²⁹ into a new discipline—sociobiology—which is the evolutionary study of all forms of social behaviour. Wilson predicts that classical ethology is destined "to be cannibalised by neurophysiology from one end and sociobiology and behavioural ecology from the other", and many would agree. Only one chapter (of 27) in his book concerns human sociobiology, but this has precipitated a vigorous debate. Wilson's thesis is that the increasingly powerful theoretical framework of sociobiology can be applied to explain human behaviour in a much more sophisticated fashion than is possible along simple darwinian lines (although evolutionary interpretations have of course been attempted). Thus sociobiologists are systematically

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reinvading such sacred temples as the human emotions³⁰ of gratitude, sympathy, indignation and guilt; traditions such as the inheritance of wealth³¹; the nature of our sexuality and pair-bonding system^{32,33}; human aggression, status, and societal fission^{29,34,35}. Wilson sees human sociobiology as a catalytic 'antidiscipline' to the social sciences¹¹, which assume human social life to be culturally determined and constrained only by the most rudimentary of biological drives. There is little doubt that simple characteristics such as neonate reflexes and facial expressions are innate products of evolution^{36,37}. Studies comparing mono- and dizygotic twins suggest genetic components in an array of traits that may be associated with the development of social behaviour¹¹. Thus without denying the importance of learning, experience and cultural evolution in shaping human behaviour, Wilson feels that underlying genetic influences can be predicted by sociobiology theory. Cultural evolution is fast compared with biological evolution^{11,38} and learning rules will be inherited rather than specific cultural forms³⁹.

Wilson's discussion of human behaviour within 'selfish gene'/sociobiology perspectives became the focus of heated attack by protagonists of environmental determinism⁴⁰⁻⁴⁵. The spearhead comes from the 'Sociobiology Study Group' of the American 'Science for the People' (SftP) association, who state "we know of no relevant constraints placed on social processes by human biology"⁴⁰. SftP attacked with considerable political fervour. They see in sociobiology more than a vestige of the 'social darwinism' that led Herbert Spencer⁴⁶ to claim that it was not "natural" to try to eradicate poverty because this interfered with darwinian processes, and which more recently reached its most appalling manifestation in Nazi atrocities. They have two major academic criticisms³²: first, sociobiology theory is so constructed as to allow no empirical tests; second, there is no evidence for human social behaviour genes, "yet sociobiologists break the totality of human social phenomena into arbitrary units, . . . postulating particular genes for each". The first point seems partly unfair because certain predictions are amenable to a degree of scrutiny, though there are admittedly problems in interpreting the evidence. However, it is true that virtually nothing is known about the existence or operation of underlying genetic influences of social behaviour. Current models are likely to prove at best naive; but to abandon them until genetic mechanisms are elucidated seems perhaps pessimistic.

Wilson⁴⁷ claims (perhaps with justification^{48,49}) that intimidation and "academic vigilantism" are operative, and that his views have been distorted. He stresses^{49,50} the need to avoid the "naturalistic fallacy" of ethics, that is the philosophy of "what is,

should be". The 'what is' concerns our genetic legacy shaped by the selective forces of the Pleistocene. However, insights into innate predispositions may help us face the future with greater insight and humanity^{50,51}. Wilson often discusses the possibilities for evolution of cooperative and altruistic traits in humans^{29,50,51}, claiming¹¹ that social darwinism died with the advent of sociobiology because this delineated the role of altruism in societies and rendered it consistent with population genetics. He retaliates to SftP, that indeed the theory of total cultural determination logically implies that human value systems will perpetuate and validate the *status quo*^{47,52} (Skinner's views of near total environmental determinism were also attacked by SftP, largely because of the political consequences he drew from his work).

There is yet another problem that affects students of innate behaviour. This concerns the extent to which selective processes can account for biological features. Gene frequency changes can be brought about not only by selection, but by mutation pressure, migration, and a sort of sampling error effect that has been termed genetic drift or the Sewell-Wright effect. If selection on alternative alleles is weak and effective population size small, it is possible for alleles to change in frequency and become fixated in the population by random chance. Another point is that certain features may be the chance result of changes in other systems; in the (arguable) absence of selection, these might remain unmodified. Furthermore, although the selective forces on different populations may be identical, drift can produce populations showing different genetic constitutions if there are multiple stable equilibria for the particular system. Thus R. C. Lewontin⁵³ has called selfish gene/sociobiology philosophy "vulgar darwinism" and "panglossian", because in it all behaviour is seen as adaptive. The question is how much of observable behaviour is the fine-tuned product of natural selection and how much is 'noise' that has, in a sense, escaped selection.

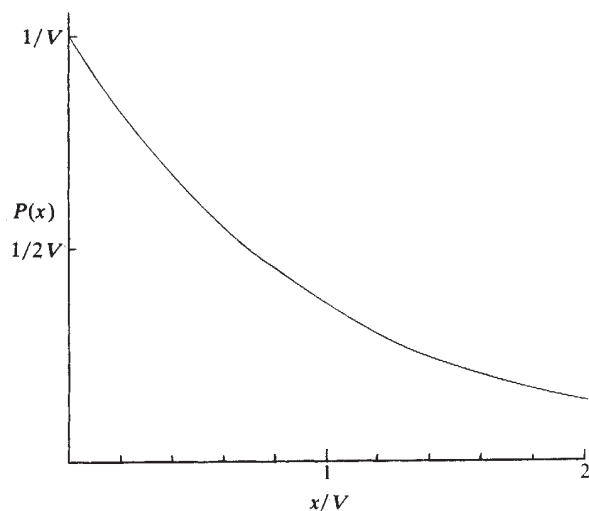
In summary, sociobiology and evolutionary ethology are developing astride at least three separate controversies: the ever-present nature versus nurture debate; the fading problem of modes of selection and the 'advantage to the species' heresy; and a controversy concerning the extent to which observable behaviour is adaptive. I will now review briefly some of the sorts of selfish gene theory that are being developed as adaptive interpretations of behaviour.

The theoretical approach: evolutionary stable strategies and behaviour

Classically, most evolutionary models of animal behaviour have been frequency-independent; that is, the strategies played by other individuals in the population are assumed to be fixed, or are irrelevant to the model. For example, a considerable part of animal behaviour concerns the gathering of essential resources from the environment (foraging). Assuming that these resources have fitness-related effects, models can be constructed to determine how selection will maximise resource uptake rate in accordance with environmental features. It is possible to construct and test a wide variety of optimal foraging models⁵⁴⁻⁵⁹ in which the 'decision' rules to be optimised concern for example optimal prey choice, optimal time to stay in a resource patch, and so on. A general approach, which seeks to predict decision rules for strategy shifts related to an animal's physiological deficits is also being developed⁶⁰⁻⁶². Essentially the decision rules depend entirely on the sets of environmental features to be harvested. Each particular condition defines a pure, optimal strategy; animals are expected to (and often do) show various forms of cue monitoring (resource assessment strategies⁵⁹) and modify behaviour in accordance with the optimal set of rules.

However, much if not most animal behaviour (and that of interest to sociobiologists) is affected either directly or indirectly by the behaviour of other individuals. The fitness benefits that accrue from a given strategy are often strongly dependent on what other conspecifics are likely to do; that is, payoffs are

Fig. 1 ESS for 'war of attrition' game. V is the value of winning and x is the display cost that an individual is prepared to pay (from ref. 66).



frequency-dependent. This poses special difficulties for population biologists. To my mind, far the most important insight for the theoretical study of sociobiology, and the one that addresses this problem, is the idea of the 'evolutionarily stable strategy' (ESS), defined and developed by J. Maynard Smith⁶³⁻⁶⁵. Roughly, a strategy is an ESS if, when most of the population show it, there is no alternative strategy that can invade and spread. Thus an ESS is an unbeatable strategy, and any mutant with different behaviour will be lost through selection. ESS solutions often rely heavily on von Neumann's⁶⁷ mathematics of games theory. An ESS can be a pure (that is a unique or 'optimal') strategy such as 'when in circumstance A, play a'; or it can be a mixed strategy—for example 'when in A, play a with probability P_a , b with probability P_b , ... and so on'.

As yet, the ESS approach is in its infancy. There are some important problems to be aware of. First, a strategy can be an ESS only within the predetermined set of rules ('game') on which the model is built—changing the set of alternative strategies may cause the ESS to change. As always, the modelling of a particular game must be judged by its plausibility for the natural situation. Second, a given game may have more than one ESS depending on starting conditions. This seems a contradiction until one remembers that ESSs are frequency-dependent. A strategy that could be unbeatable if others all play it may not be able to invade into a population that is fixed for another strategy. Thus some ESS analyses tell us rather little (at least superficially) about which ESS will evolve; they merely indicate what the ESSs are. This aspect of ESS theory might be developed further. Selfish-gene population genetics theory indicates the direction of movement from the initial starting conditions to an ESS. If one can deduce the probable starting conditions, it will be feasible sometimes to deduce the most likely 'game ontogeny' and 'ESS succession'. The ESS for an earlier game sets the starting conditions for the next, more complex game (as adaptive subtlety increases), this determines the next ESS and so on. Third, some games have no ESS, instead they result in unresolvable evolutionary chases⁶⁸ with cycling frequencies of strategies (limit cycles⁶⁹). Fourth, there is the problem of genetic mechanism for complex, mixed ESSs. There can be theoretical difficulties if one postulates that a mixed ESS distribution corresponds to genetic polymorphism of component pure strategies⁶⁶. The more feasible alternative might be that individuals are genetically monomorphic, each individual playing the component pure strategies with the ESS probability distribution⁶⁶. Finally, though unbeatable, an ESS is not necessarily the strategy that appears to be 'best' intuitively—even using selfish gene optimality predictions. For example, classical (frequency-independent) models often predict the strategy with the maximum initial rate of spread into a parental population of deduced constitution. These optima are often not ESSs.

So far, the main application of ESS theory to behaviour has concerned animal fighting⁶³⁻⁶⁶; one or two examples can be outlined. In the celebrated 'war of attrition' game^{65,66} two opponents contest ownership of a resource worth V fitness units by displaying to each other. There is no physical damage, the winner is the opponent that is prepared to display longest. The costs ($-x$ fitness units) of the dispute are related to time and energy spent on display; thus a strategy concerns the choice of cost level $-x$. In fact there is no unique x value that is an ESS for this game, the ESS (a mixed one) is to play variable x with probability:

$$P(x) = \frac{1}{V} \exp(-x/v) \quad (1)$$

Thus individuals should vary in their persistence after the negative exponential distribution shown in Fig. 1. In nature, many display durations (and other behaviours) follow negative exponential distributions, but it is not yet clear whether any obey (1).

In the 'hawks and doves' game⁶⁶, it is physical damage that decides the outcome. There are two alternative strategies: 'hawk' is prepared to fight at an escalated level and will retreat

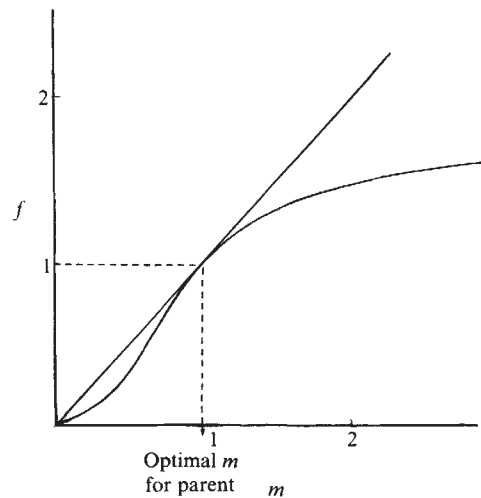


Fig. 2 Optimal amount of parental investment (PI) for parent to invest in each offspring. Viability and/or reproductive success f of the offspring are assumed to be some increasing function of m , the amount of PI. The optimal value for the parent has $m = f(m)/(df/dm)$; given by the tangent shown.

only if damaged (cost $= -D$ fitness units); 'dove' retreats immediately (without damage) if its opponent escalates. If two doves meet, each is equally likely to win, without cost. The ESS is to show the hawk strategy with probability $P = V/D$, that is play pure hawk if $V > D$ but a mixed hawk/dove strategy if $V < D$. Behaviour such as elephants show in 'must' could be relevant to this model.

Both these games and various others⁶³⁻⁷² are symmetric (conditions identical for each opponent). This is clearly very simplistic; in nature asymmetries will always exist and this can profoundly influence the ESS^{65,66}. Often the ESS will be to permit the asymmetric cue to settle the contest conventionally (that is without escalation or display costs), especially when opponents can obtain exact information about the asymmetry. Suppose that contests involve a prior-resident or 'owner' A and a 'newcomer' B. There are two conventional ESSs. The first is, 'when A, play cost M ($M > V$); when B play O'. The other is the reverse, 'when B, play M ; when A play O'. Both are equally probable if there are no other asymmetries, but the 'owner wins' convention is perhaps commonest in nature^{59,66,73,74}. This could relate to payoff V asymmetries between contestants, or to differences in their 'resource holding potential' (RHP)^{66,74}. However, the 'owner wins' convention is also more likely for another reason. It establishes a definite advantage for newcomers to search for alternative resources. A 'newcomer wins' convention may be vulnerable to invasion by a mutant strategy in which the displaced owner retreats some way, then presents himself again in the guise of newcomer.

Strong evidence that owners do escalate against breaches of 'owner wins' conventions have been found in insect territoriality^{59,72,73}. Many humans succumb to the temptation to escalate when faced with a 'queue-jumper', or act of trespass, or attempted mate-stealing!

In the owner/newcomer case the asymmetry is (at least superficially) uncorrelated with differences in payoff or RHP. Asymmetries in payoff or RHP each similarly yield two ESSs; these can be of a 'commonsense' variety (that is 'opponent with higher payoff wins' or 'stronger opponent wins') or of a 'paradoxical' type ('opponent with lower payoff wins' or 'weaker opponent wins')⁶⁶. However, 'commonsense' ESSs are generally more likely to evolve than paradoxical ones, and in some cases only the commonsense ESS can evolve from the likely initial ESS that ignores the asymmetry⁶⁶. In general, animal conventions obey 'commonsense' patterns⁷⁴.

Some of the most impressive developments of sociobiology theory have involved the field of intra-familial interactions. Kin selection¹⁵ theory now offers a valuable explanation of the

evolution of apparently altruistic behaviour between related individuals. The idea that selection can favour apparent altruism, in which individuals sacrifice some of their own reproductive success to enhance that of relatives goes back to Fisher⁷⁵ and Haldane⁷⁶ who were concerned with the evolution of sterile castes in insects. But until the classic work of W. D. Hamilton⁷⁷ the concept remained undeveloped and was ignored by ethologists. Hamilton suggested that selection will favour evolution of altruism provided that ratio k (=fitness benefit to a group of relatives/cost to self) satisfies

$$k > \frac{1}{r} \quad (2)$$

in which r is a measure of the degree of relatedness between the individuals concerned. Thus $r = \frac{1}{2}$ for full sibs under diploidy, and $\frac{3}{4}$ for full sister sibs under haplodiploidy. The higher the value of r , the lower the k ratio required to satisfy condition (2), and hence haplodiploid systems (notably hymenoptera) might be highly predisposed to evolve altruisms such as worker sterility. There is a vast literature^{29,78} relating observed behaviour to kin selection.

Altruism through kin selection is not in conflict with 'selfish gene' philosophy—a hypothetical allele for altruism spreads because it enhances its own replication through other individuals. Confusion has arisen because Hamilton's models were not standard gene-frequency ones. Thus, much discussion (often leading to errors of deduction) has occurred along the lines of what a full sib or half sib is 'worth' from the point of view of 'self' (for example $\frac{1}{2}$ or $\frac{1}{4}$ respectively). This completely obfuscates the fact that it is the probability of sharing the altruism allele that is important; this depends on gene frequency. There have been population genetics approaches to kin selection^{79–85}; in particular Charlesworth⁸⁵ has recently made major developments by analysing the crucially important effects of (environmentally

or randomly determined) probability of expression of the altruistic gene in sibships. Imagine a rare dominant mutant allele for altruism. The progeny of a mutant heterozygote parent will be half non-altruist homozygotes, and half heterozygote carriers of the allele. Clearly, if the allele has a very high probability of expression, then only the non-carriers of the altruism allele will receive benefits, and altruism is likely to be lost whatever the k value. Spread of the allele becomes possible only as the probability increases that beneficiaries are also carriers; Charlesworth derives ESS values for the ratio of altruists to non-altruists among carrier sibs, expressed as a function of k . Ultimately ESS approaches based on population genetics are likely to replace the (less rigorous) classical 'inclusive fitness'⁷⁷ approach to kin selection, which generally yields only minimum values of k for evolution of altruism between sibs⁸⁵.

Intra-familial altruism might also evolve by a process of 'parental manipulation'^{86,87}, in which parents are assumed to be adapted to produce progeny within an optimal arrangement of altruistic tendencies so as to maximise the number of grandchildren. This is not a new idea; the late H. E. Hinton often invoked it, though sometimes failing to stress that beneficiaries must be related to donors. Charlesworth⁸⁵ has shown that the necessary k values to allow spread of altruism alleles are invariably lower for parental manipulation than for kin selection and ESSs are correspondingly higher, often permitting full expression of altruism by carriers.

The parental manipulation idea poses the problem (stressed by R. L. Trivers) of a possible conflict of evolutionary interest between parents and their offspring^{77,87}. In sexually reproducing diploids, a trait can evolve in an offspring for garnering more parental investment (PI (ref. 88)) than is optimal for its parent^{87,89,90}. Assuming that viability and reproductive success f of each offspring is increased by the amount m of PI it receives, the optimal m for the parent (through numbers of grandchildren) is given by the tangent to $f(m)$ from the origin^{89,91,92} (Fig. 2), that is it occurs where

$$m = f(m) / \frac{df}{dm}$$

For convenience, m and f are expressed in terms of factors of the parental optimum, at which $m = 1$ and $f = 1$. Using population genetics models, we^{89,90} were able to show that there will be ESS levels of conflict in sibships, depending on the mating system. For full sibs (or half sibs with equal PI for the sexes) the ESS m^* is given by

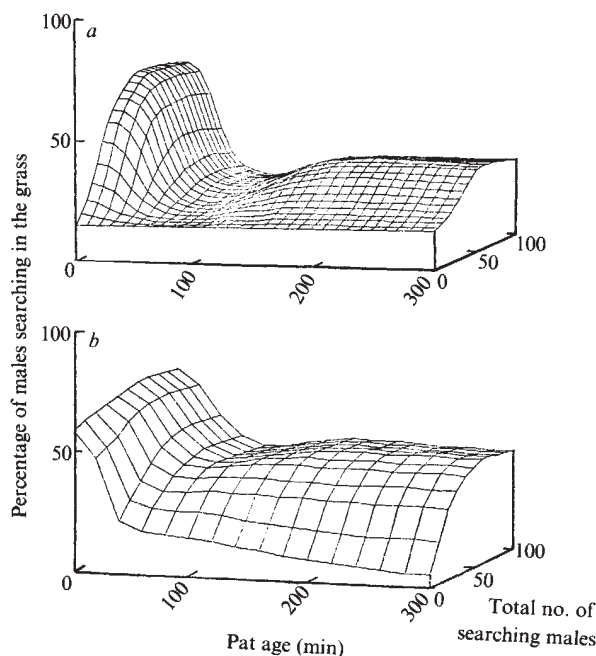
$$m^* = f(m^*) / \frac{2df}{dm^*}$$

and for half sibs with zero male PI the denominator becomes $4(df/dm^*)$. The ESS for all diplo-diploid mating systems must be between these limits. As with altruism, attempts to deduce the nature of parent-offspring conflict along classical kin selection lines have sometimes led to incorrect deductions (compare conclusions of ref. 93 with ref. 89), reinforcing the assertion that intrafamilial evolutionary games are best solved by a combination of standard population genetics and ESS approaches.

A particularly tantalising problem in sociobiology concerns the evolution of mating systems^{85,94–96}. In a sense, this reduces to the question of how much PI each sex gives to its offspring. Whether it is advantageous for a male or female parent to invest care in offsprings or to desert depends on what the other partner is likely to do, on the offspring survival prospects with 0, 1, or 2 parents investing, and on the value of desertion in terms of finding new mates. ESSs for PI games of this sort have been examined by Maynard Smith⁹⁷, who makes plausible deductions about which ESS is likely to evolve in relation to such features as internal versus external fertilisation.

Sexual conflict⁶⁸, in which the evolutionary interests of the two sexes is opposed, is inherent in the evolution of mating systems and is a common product of intra-sexual competition.

Fig. 3 *a*, predicted profile for male dungfly search strategy, assuming an 'ideal free' distribution (equality of fertilisation rates) between searching on the dropping and searching in the surrounding grass. The profile shows the expected proportion of males to be found in the grass, plotted in relation to the variables of dung age and total number of searching males present. *b*, Observed profile, from field data. This has been averaged for certain age groupings of droppings and will thus be smoothed out to some extent. The predicted and observed profiles are very similar, indicating that males are adapted to respond in a complex manner to cues correlating with pat age and competitor density (from ref. 99).



An example concerns mating decision. It is sometimes of selective advantage in a given sexual encounter for the male to mate but for the female not to mate. Cases could include some conditions of: optimal mate choice, incest, and meetings of males with mated females or those of a different race. This game is a strongly asymmetric one, both in payoffs (value to male of mating, value to female of not mating) and in cost functions (for example cost of attempting rape against cost of resistance). Simulations of the 'war of attrition' type yielded the expected 'conventional' outcomes in which one sex plays 0 with the other prepared to persist to some value greater than its opponent's payoff⁶⁸. However, suppose that outcome is determined by size or some similar feature, and that this imposes a fitness cost that acts independently of the play of the opponent. Thus increasing size increases prospects of winning, but decreases fitness in some other respect, independent of the present game. Simulations here yielded 'unresolvable evolutionary chases' (no ESS)⁶⁸.

Extent of behavioural adaptiveness

The only way that sociobiologists can resolve the 'behavioural adaptiveness' controversy is by precise empirical tests of their models. It is often very difficult to relate behaviour (directly or indirectly) to fitness costs and benefits, measured as reproductive success. I will outline a study in which direct fitness-costings of behaviour strategies are possible, and where the undeniably complex demands of the models might be seen as 'naïve pan-selectionism' were it not for the fact that the observed behaviour fits the predictions. The model concerns competitive searching in systems where there is no guarding of resources. The prediction is that stable spacings called 'ideal free distributions' will evolve; that is mechanisms of spacing of animals in relation to resource quality and competitor density, such that all individuals achieve equal payoffs through time and space^{57,59,99-101}. This applies when fitness prospects decline with increased competitor density in the various alternative resources within the habitat. The study in question concerns competitive mate-searching strategies shown by male dungflies, *Scatophaga stercoraria* L.

Male dungflies search on or around cow pats for females, which arrive to mate and lay their eggs. After mating, males guard their females during oviposition. During the first 20 min after the dung is deposited, males are distributed in apparent ideal free fashion around the mating system as judged by the captures of incoming females (Table 1)⁹⁹. Males can also achieve gains through 'takeovers' of paired females, and this becomes significant later in the life of a dropping. (Exact egg gains from takeovers can be predicted from sperm competition experiments¹⁰²). Consider two strategies: searching on the dung surface, and searching in the surrounding grass. Gains through takeovers arise almost exclusively from the former, but gains through matings with incoming females may be higher in either place, depending on competitor density. At high male densities, most incoming females are caught in the grass. Two variables thus prescribe an ESS ratio of searching males in the two places:

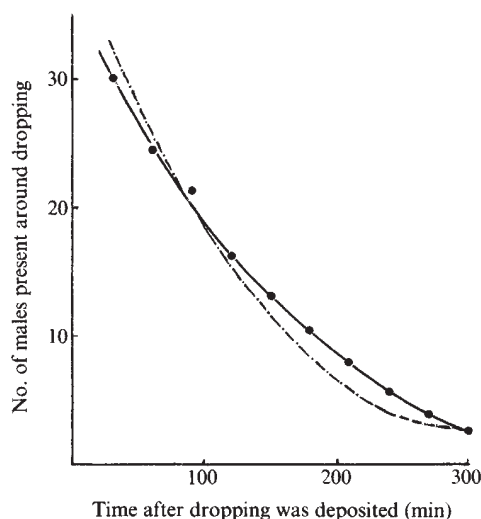


Fig. 4 Observed decline (heavy line) in mean number of male dungflies at a cattle dropping compared with the decline predicted by 'input matching law', equation (3) (light line). The predicted curve is corrected to account for the time males spend with females. The fit is quite close, suggesting that the ESS (3) is obeyed (from ref. 59).

age of dropping (because this fixes the proportions of incoming to paired females) and total male density. The predicted ESS profile (computed from a large amount of field data and sperm competition analysis) is close to the profile obtained by field observation (Fig. 3).

Superimposed on these spatial ESSs, there is also a complex temporal ESS. The rate of female arrivals (dG/dt) declines with age of dropping. Each male gains $1/n$ of the input; individual gain rates are thus $(1/n)(dG/dt)$. The temporal ESS is a mixed one in which over a certain time range (the 'emigration range') males should show a 'phased withdrawal' so that the number n present at time t follows an 'input matching law'⁵⁷

$$n(t) = \frac{1}{SL_{\max}} \left(\frac{dG}{dt} \right) \quad (3)$$

Constant $SL_{\max}$ is the mean female capture rate per male, for the entire time spent searching. If this ESS is obeyed all males have equal gain rates irrespective of their departure times; evidence suggests that it is^{57,98} (Fig. 4).

Achievement of ideal free stability in the dungfly mating system thus implies that we should become more rather than less optimistic about the level of adaptive behavioural complexity. When behaviour strategy can be costed adequately in terms of fitness, it is apparent that animals exhibit response surfaces of considerable adaptive complexity.

Table 1 Observed and predicted numbers of female dungflies caught by males in each of a series of zones around cattle droppings

Zone around dropping	Summated males in each zone	Proportion of males	Observed female captures in each zone	Proportion of female captures	Predicted female captures in each zone
On dung surface	5,575	0.35	132	0.36	127.9
Surrounding grass band up to 20 cm from dung edge	7,303	0.46	178	0.49	167.8
Band 20-40 cm from dung edge	2,196	0.14	41	0.11	50.3
Band 40-60 cm from dung edge	641	0.04	12	0.03	14.7
Band 60-80 cm from dung edge	227	0.01	4	0.01	5.2
Total	15,942		366		365.9

The total males present were summed for each zone at the time of every female capture. If an 'ideal free' distribution holds all males should have equal prospects and so the proportion of males searching in each zone should equal the proportion of females captured there. This appears to be the case (from ref. 99).

And man?

A major weakness of current sociobiology theory is the omission of insights into evolutionary dynamics of animal learning; there is little or no formal modelling of the learning process. It is adaptive mainly as a mechanism for optimising strategy in a changeable environment. The act of reinforcement itself must be an evolved phenomenon and reward or punishment value must relate to 'fitness change effect', with time and effort spent in absence of reward acting as negative reinforcement. The aim (set by selection) is thus to maximise 'net positive reward'; that is, net positive fitness change. Motivation can be seen as the evolved mechanism underlying adaptive strategy and reward value shifts in relation to internal cues of physiological deficit and external cues associated with resource availability.

With major lacunae such as this, it is obvious that some forays into human sociobiology will appear naïve. In man, goals and rewards are often indisputably set by the cultural environment, though underlying structures of the acquisition process and its consequences are arguably innate. An appropriate analogy might be that we receive basic equations by genetic bequest, but that the values of parameters within them are largely fixed by culture. Thus, ultimately programmed by evolution, we can resolve towards helpful altruism or its opposite depending on prevailing social mores. Our behaviour will always be under scrutiny; this will have been a major selective force. Even our fantasies need not be chance byproducts of brain function; a sociobiological view could be that they represent 'long shot' mental scanning of fitness-related contingencies.

Both genetic and environmental determinism tend to predict unswerving cultural stability. Culture groupings can represent anything from the totality of society down to one of its component idea-groups. If indeed fitness in the Pleistocene did relate to status in a sub-group²⁹, then a sociobiological model for cultural change might resemble a game related to status enhancement in as large an idea group as possible, but which in certain conditions generates advantages in a risky process of establishing (and recruiting to) new idea-groups. Cultural change would thus represent the cumulative manifestation of a sort of frequency-dependent, self-generating mechanism for societal turbulence.

Hopefully, few would deny that testable models of animal sociobiology have an importance for the developing science of animal behaviour. If there is a cause for pessimism about the scientific fate of human sociobiology, it is because precise empirical tests are generally impossible, though endless debate will occur over interpretations of evidence. It is not yet clear that we have adequate techniques to make useful progress in the struggle to disentangle our nature from nurture; Feldman and Lewontin¹⁰³ have cogently argued the difficulties of applying heritability analysis to human behavioural characteristics. My own prejudice concerning the extent of genetic predisposition ranges from enthusiastically positive for features like emotions³⁰, to reserved for cultural and social structure. Even if emotions like gratitude, indignation, sympathy and guilt do prove to have strongly innate bases, this seems no reason to devalue them.

The eminent sociobiologist R. D. Alexander, discussing underlying selfishness, suggested that "Selection has probably worked against the understanding of such selfish motivations becoming a part of human consciousness, or perhaps being easily acceptable"¹⁰⁴. I can personally testify to an aversion to statements such as that made by Ghiselin¹⁷: "Yet given a full chance to act in his own interest, nothing but expediency will restrain him (man) from brutalising, from maiming, from murdering—his brother, his mate, his parent or his child". If I can admit to a fear about human sociobiology, it concerns what Lewin¹⁰⁵ terms "biological limits to morality" and ultimately to avoidance of the "naturalistic fallacy"¹⁰⁶. There are difficulties. For instance, if men are shown to have genetically-based shorter lives than women, it seems appropriate to consider this in a discussion of retirement age. Failure to heed genetic fact can here cause

unfairness. But suppose it were proven that I am genetically predisposed to bequeath all possessions to my son at my daughter's expense³¹. In this case, unless it were unequivocally established that an egalitarian distribution causes asymmetric hardship, I would not wish to heed genetic fact. Genetic predisposition itself seems irrelevant to 'right' decisions; the important question is whether ignoring a predisposition can cause injustice.

Faced with facts, we can perhaps make equitable judgements. But what of hypotheses? These could lead us to a fairer world or to a nightmare of inhumanity, depending on their correctness. Wilson says "One does not efficiently eliminate injustice stemming from a genetically based human nature by denying the existence of that nature or prohibiting research on it"¹⁰⁷. SftP argue that it is incorrect assumptions about human nature that have led to the injustices of the present. Both points of view seem to me to argue for continued research into human nature, though I am less than sanguine about immediate prospects of progress. It is certainly best that we proceed with integrity and caution.

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articles

The origin of ozone in the troposphere

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Examination of the distribution of tropospheric ozone indicates that surface destruction in the Northern Hemisphere (NH) should be about three times larger than in the Southern Hemisphere (SH). If, according to the traditional understanding of ozone, this species were a passive tracer in the troposphere, a threefold larger flux out of the stratosphere should exist in the NH than in the SH. However, meteorological analyses fail to support such pronounced hemispheric differences in stratosphere–troposphere exchange. Alternatively, therefore, we hypothesise that photochemical synthesis of ozone in the troposphere may be particularly important in the NH because of asymmetries in the sources and distribution of carbon monoxide, hydrocarbons and nitric oxide.

EXCEPT around urban centres during pollution episodes, it is commonly assumed that tropospheric ozone originates from the stratosphere, is chemically inert, and is destroyed by contact at the Earth's surface (see, for example, refs 1 and 2). This traditional understanding of the origin of tropospheric ozone is superficially supported by observations that average

ozone volume mixing ratios increase with altitude up to a maximum at 30 km. Furthermore, a distinct gradient of ozone concentrations usually exists in the boundary layer with low concentrations at the surface. The observations of tropopause folds which transport large quantities of ozone downwards to the ground on a relatively short time scale^{3,4} similarly support the hypothesis that injection from the stratosphere is a major source of tropospheric ozone. Nevertheless, despite this observational evidence which favours a stratospheric origin of tropospheric ozone, it is possible to argue the possibility of significant tropospheric ozone production.

Ozone distribution

In particular we wish to compare the hemispheric asymmetry in the observed land mass and ozone distributions. Because ozone destruction is about 10 times greater over land surfaces than over oceans⁵ and because higher ozone concentrations are observed in the Northern Hemisphere (NH) troposphere despite a larger land mass⁶, a simple calculation (see Table 1) shows that ozone destruction at the ground in the NH should be approximately three times larger than in the Southern Hemisphere (SH). To derive the flux estimates given in Table 1, we have used Aldaz's deposition rates of 0.6 cm s⁻¹ over land, 0.04 cm s⁻¹ over ocean, and 0.02 cm s⁻¹ over snow⁵. Thus, to account for this difference in the destruction rate at

the Earth's surface, the assumption of inert tropospheric ozone requires the flux of stratospheric ozone into the troposphere of the NH to be at least three times larger than in the SH^{7,8}. Taking into account the estimated transport of 5.3×10^{35} molecules of ozone per year from the NH to the SH⁷ this difference becomes more than a factor of four. To see whether this assumption is supported by observations, we focus our attention on the available observations of ozone and various meteorological indicators of stratosphere-troposphere exchange processes. Although it has been claimed that radioactive fallout observations favour a two to three times larger troposphere-stratosphere exchange in the NH than in the SH⁸, the arguments in favour of this statement were quite limited.

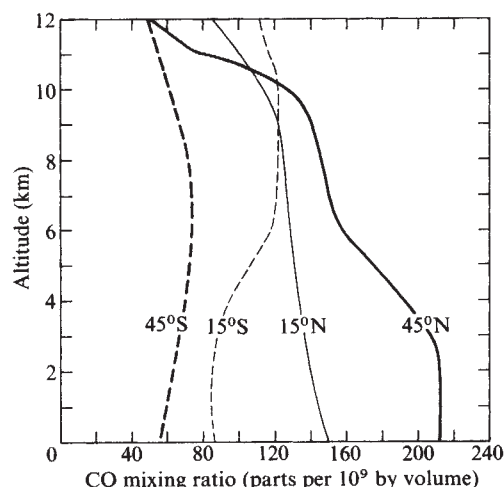


Fig. 1 Tropospheric profiles of carbon monoxide at representative latitudes which show the strong asymmetrical hemispheric distribution of this gas (redrawn from the analysis of Seiler²¹).

Dütsch⁶ has provided a detailed analysis of the average distribution of ozone as a function of latitude and month for both hemispheres below the 10 mbar level (~ 30 km). His data show that the seasonal and height distributions of ozone within the two hemispheres are far from sufficiently different to explain a flux of ozone from the stratosphere which is three times stronger in the NH. The greatest difference between the two hemispheres is observed polewards of 65° , where a difference of as much as 30% is seen during the spring seasons of the two hemispheres. Differences observed at these high latitudes would be important only if most of the exchange between the stratosphere and troposphere would take place at these latitudes in the NH. However, no evidence to support this contention is available from an analysis of either the meteorological fields or radioactive fallout⁹. In mid-latitudes, where a better data base exists, we note the similarity of both the seasonal variability and distribution of ozone in the two hemispheres above the tropopause. The analyses of Dütsch⁶ and Pittcock¹⁰ indicate that there is very little difference in ozone concentrations between Aspendale (38° S) and Boulder/Albuquerque (37° N) at the 40 and 200 mbar pressure levels. Therefore, an examination of the meteorological parameters which are indicative of the intensities of stratosphere-troposphere exchange would have to provide evidence of considerably stronger stratosphere-troposphere exchange in the NH than in the SH.

Tropospheric-stratospheric exchange

The mechanism by which stratospheric air is brought into the troposphere is an issue which is currently debated. Whereas Newell⁹ and Danielsen and Mohnen⁴ argue that most of the stratospheric ozone enters the troposphere in the region of baroclinic zones and tropopause discontinuities, Reiter¹¹

claims that mean meridional motions are also important in the transport of stratospheric ozone into the troposphere. Presupposing that either exchange mechanism may be correct, we examine whether the meteorological observations might be consistent with a more than three times larger influx of stratospheric ozone in the NH.

A measure of air exchange through the tropopause folds must be deduced indirectly from meteorological observations which are indicative of this mechanism. Since Danielsen and Mohnen⁴ state that most of the mass exchange takes place during large-scale cyclogenesis, we first compare the cyclonic activity in the two hemispheres to determine whether or not this process is significantly stronger in the NH. Taljaard¹² has analysed the general meteorological synoptic patterns in the SH and has provided a comparison of the cyclonic frequency of the two hemispheres. He concludes that the preferred latitudes for cyclones in both hemispheres are similar and that the frequencies of occurrence are comparable. Integrating the total cyclonic frequency over each of the hemispheres shows that cyclones occur about 10% more often in the SH. Excluding the summer tropical cyclones of the SH (since no stratosphere-troposphere exchange takes place during these events), the cyclonic frequencies of the two hemispheres are nearly identical.

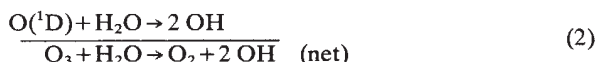
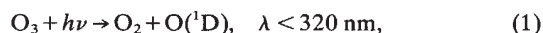
Even with almost equal cyclonic frequencies, the argument can still be made that exchange processes are more dominant in one of the hemispheres if the cyclonic intensity in that hemisphere were significantly larger. To determine this possibility, we examined the standard deviation of the geopotential of the 500 mbar pressure level. The relative measure of this quantity is indicative of the migration of the deep troughs and ridges associated with cyclone passage. Van Loon¹³ analysed this quantity as a function of both latitude and season for both hemispheres, finding that the standard deviation is considerably greater polewards of 30° in both hemispheres. Integrated throughout the entire year, the average standard deviation is slightly higher (7%) in the SH. Thus a more than three times larger average ozone flux into the NH troposphere cannot be supported by an analysis of such meteorological parameters which are most indicative of large-scale cyclonic activity.

According to Reiter¹¹, the mean meridional circulation in the tropics is responsible for much of the exchange of mass between the troposphere and stratosphere. Thus, large hemispheric differences in the mean meridional circulation could provide a larger flux into the NH. Examination of these wind patterns in the tropics for each of the seasons¹⁴ shows that the descending branch of the Hadley cell (between 20° and 30°) is most pronounced during the winter season of each hemisphere. However, the average downwards motion in the SH winter is slightly larger than the corresponding term in the NH and we conclude that the mean meridional tropical motions are not responsible for transporting a significantly larger quantity of ozone into the NH troposphere.

Photochemical source

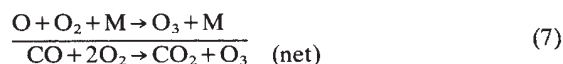
Altogether, despite some admittedly severe limitations in the available data base, we conclude that there is no convincing meteorological evidence to support drastic differences in the stratosphere-troposphere ozone exchange rates occurring in the two hemispheres, especially at midlatitudes, where exchange should be maximum. Thus it is reasonable to postulate that the asymmetry of the tropospheric ozone source strengths in the two hemispheres could be caused by photochemical processes in the troposphere. We contend, therefore, that there may be an important *in situ* contribution to the tropospheric ozone budget from relatively slow, but steady, photochemical processes taking place throughout the entire volume of the troposphere. Crutzen^{15,16} suggested that photochemical reactions produce and destroy appreciable amounts of ozone in the non-urban troposphere and that these quantities cannot be neglected in the formulation of a global tropospheric ozone budget. Using a one-dimensional numerical model which incorporated both transport and photochemical

processes, Fishman and Crutzen¹⁷ showed that about half of the ozone transported through the tropopause could be destroyed photochemically in the troposphere before reaching the ground through the reaction pair:



Note that the amount of ozone destroyed by this mechanism is dependent only on the amount of water vapour present in the atmosphere and two reaction rates which are fairly well established. In addition, ozone loss occurs through the reaction $\text{HO}_2 + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$. Altogether, we estimate a 1–2 month tropospheric residence time against gas-phase destruction during summer¹⁷. However, the hydroxyl radical (OH) formed by reactions (1) and (2) may react with methane and carbon monoxide to initiate catalytic cycles which produce ozone in the troposphere. The amount of ozone formed by these processes can be estimated to be greater or smaller than the amount destroyed photochemically depending on certain reaction rate constants and the prescribed background concentrations of NO and NO₂, which currently are not well-established. Thus, Fishman and Crutzen concluded that these uncertainties hindered quantification of the relative importance of photochemistry in the tropospheric ozone budget and that the debate over the origin of tropospheric ozone cannot be resolved^{18–20}. Although the oxidations of methane and other hydrocarbons may likewise provide important sources of tropospheric ozone, we will focus the present discussion on the photochemical oxidation of CO since its chemistry seems well known.

The chain of reactions most likely to occur in the troposphere resulting in the production of ozone goes as follows:



We note here also that reaction (5) competes with the reaction $\text{HO}_2 + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$ and thus prevents the loss of ozone.

The most comprehensive analysis of the global carbon monoxide cycle has been carried out by Seiler²¹. In Fig. 1, we show four representative profiles taken from his data which depict the strong latitudinal gradient in mixing ratios. Integrated throughout the entire globe, we note that there is approximately twice as much carbon monoxide in the NH. Although Wofsy²² and Crutzen and Fishman²³ suggested that more OH should be present in the SH because of the smaller CO concentration there, the higher tropospheric ozone concentrations (see Fig. 2) and NO concentrations²³ in the NH offset the CO asymmetry sufficiently so that nearly equal diurnally and seasonally averaged OH concentrations, most likely ranging between 3 and $9 \times 10^5 \text{ cm}^{-3}$, are calculated in both hemispheres. Note that our tropospheric ozone analysis is quite consistent with previous studies^{6,11}. We were concerned with the possible biases which may have existed in the ozone-sonde analyses at various sites, especially in the tropics where ozone data are scarce and where we calculate the highest OH number densities. It is in this region that our analysis indicated 26% more tropospheric ozone between 0° and 30° N than between 0° and 30° S . However, the surface data taken from the German project *Troposphärisches Ozon* which utilises the same type of automatic recording ozone instruments at every station in this network (between 70° N and 34° S) also exhibit a similar difference (31%) in the tropical regions of the two hemispheres⁷. Measurements by Rasmussen *et al.*²⁴ taken during a Pacific Ocean voyage show the presence of about 50% more ozone between 0° and 15° N than between the equator and 15° S . Furthermore, on the basis of the known source strengths of NO_x in the world^{25,26}, we have prescribed the total amount of NO_x (NO + NO₂) in the NH to be 60% greater than in the SH for these calculations. We note that the assumed tropospheric NO_x column densities in the two hemispheres ($3.4 \times 10^{14} \text{ cm}^{-2}$ and $2.1 \times 10^{14} \text{ cm}^{-2}$) are lower than Noxon's²⁷ estimated clean air upper limit of $5 \times 10^{14} \text{ cm}^{-2}$ for the western NH. This corresponds to an average concentration of less than 0.1 parts per 10^9 by volume in the troposphere. However, even the lowest calculated average concentrations of OH ($3 \times 10^5 \text{ cm}^{-3}$) allow $9 \times 10^{14} \text{ g}$ of CO per yr to be oxidised in the NH and $5 \times 10^{14} \text{ g}$ per yr in the SH. If there were one ozone

Table 1 Hemispheric ozone flux calculations

Latitude belt:	0°–15°	15°–30°	30°–60°	60°–90°	Hemispheric total
Northern Hemisphere					
Average concentration*	4.0×10^{11}	6.0×10^{11}	6.1×10^{11}	5.0×10^{11}	
Hemispheric fraction covered by†:					
Land	0.06	0.08	0.18	0.08§	0.40
Sea	0.20	0.16	0.18	0.06	0.60
Flux over land‡	1.4×10^{10}	2.9×10^{10}	6.6×10^{10}	0.1×10^{10}	11.0×10^{10}
Flux over sea	0.3×10^{10}	0.4×10^{10}	0.4×10^{10}	0.1×10^{10}	1.2×10^{10}
Hemispheric total for Northern Hemisphere 12.2×10^{10}					
Southern Hemisphere					
Average concentration	2.5×10^{11}	3.1×10^{11}	6.0×10^{11}	6.2×10^{11}	
Hemispheric fraction covered by:					
Land	0.06	0.06	0.02	0.06§	0.20
Sea	0.20	0.18	0.34	0.08	0.80
Flux over land	0.9×10^{10}	1.1×10^{10}	0.7×10^{10}	0.1×10^{10}	2.8×10^{10}
Flux over sea	0.2×10^{10}	0.2×10^{10}	0.8×10^{10}	0.2×10^{10}	1.4×10^{10}
Hemispheric total for Southern Hemisphere 4.2×10^{10}					

* Average ozone concentration in lowest kilometre above surface (molecules cm^{-3}).

† Taken from Aldaz⁵.

‡ Flux in units of $\text{molecules cm}^{-2} \text{ s}^{-1}$.

§ Complete snow cover assumed for these regions.

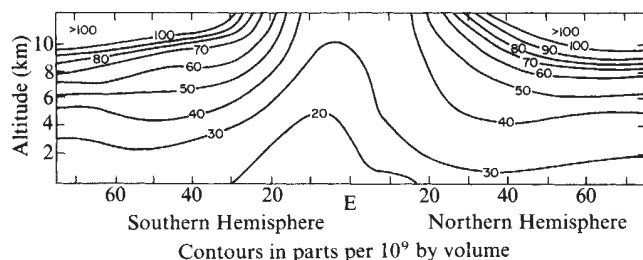


Fig. 2 Average annual distribution of ozone in the troposphere as analysed from ozonesonde data²⁹. The hemispheric asymmetry can best be detected by noting the position of the 20 and 30 parts per 10^9 contours relative to the Equator (E).

molecule produced for each CO molecule oxidised, the tropospheric ozone source strengths would be $2.4 \times 10^{11} \text{ mol cm}^{-2} \text{ s}^{-1}$ and $1.3 \times 10^{11} \text{ mol cm}^{-2} \text{ s}^{-1}$ in the NH and SH, respectively. These numbers are more than three times larger than the surface destruction rates, 0.7×10^{11} and $0.4 \times 10^{11} \text{ mol cm}^{-2} \text{ s}^{-1}$ as estimated by Fabian and Pruchniewicz². Thus, we turn our attention now to the catalytic cycle described by reactions (3) to (7) to examine whether it is realistic to assume that a significant proportion of the CO oxidised by OH-attack will lead to ozone production.

The key reaction in this cycle is reaction (5). Based on our present knowledge of tropospheric photochemistry, we can safely say that all other reactions within the chain will occur nearly 100% of the time. However, because of the extremely fast rate coefficient of reaction (5), as measured by Howard and Evenson²⁸, we estimate, using a comprehensive photochemical model and recommended rate constants, that NO volume mixing ratios must be below 10^{-11} in the troposphere before other reactions, especially the reaction $\text{HO}_2 + \text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$, dominate the fate of the hydroperoxyl (HO_2) radical²⁹. Such low concentrations of nitric oxide have rarely been observed in the NH in which there are industrial NO sources³⁰, and thus it seems that the number of moles of CO oxidised and ozone produced are nearly the same in the NH.

The preceding discussion has focused only on the possibility that oxidation of CO is a major production mechanism for ozone in the troposphere. However, it is also likely that the oxidation of methane¹⁵⁻¹⁸ and more reactive, but less abundant non-methane hydrocarbons, contributes significantly to the tropospheric ozone budget. For example, Chatfield and Rasmussen³¹, from observations at a rural site, report that urban plumes in which ozone is produced contain anthropogenic hydrocarbons. They estimate that approximately one ozone molecule is produced per emitted carbon atom. The estimated technological source strength of these hydrocarbons is about 25% (by weight) of the CO source³². The higher reactivity of these gases, coupled with the simultaneous emission of NO and NO_2 suggests that oxidation of the non-methane hydrocarbons may contribute as much as 50% ozone as provided by direct CO oxidation.

Vertical ozone distribution

It is now interesting to return to Pittcock's comparison¹⁰ of the vertical ozone distributions between Boulder/Albuquerque (37°N) and Aspendale (38°S) and to consider the almost exact agreement in ozone concentrations down to the 200 mbar level. We notice how spring maximum at 200 mbar in the NH is followed after 4 months by a pronounced maximum at 800 mbar, while the much weaker ground level maximum in the SH is only delayed by 1–2 months. A delay by 1–2 months would be more consistent with the observed maximum in ground level radioactivity fallout which takes place in April–May in the USA³³. Although transfer of ozone from the upper troposphere to ground level is expected to be slower than that of radioactive material, which is removed by rain scavenging, it

is hard to understand how the delay can be as long as 2.5 months, especially in view of the Aspendale observations showing only a 1–2 month delay between the ozone maxima at 800 and 200 mbar.

A tentative alternative explanation for the observed differences in the annual cycle at 800 and 200 mbar at the two stations may be that substantial ozone production takes place in the Northern Hemisphere during summer time, which is more than enough to offset the destruction taking place at the ground and in the troposphere. Photochemical loss processes and transport from the stratosphere more realistically describe the ozone behavior in the SH at 800 mbar.

We have put forward arguments favouring a substantial influence of *in situ* reactions on tropospheric ozone. Through formation of OH by reactions (1) and (2), tropospheric ozone exerts a substantial influence on the chemical composition of the atmosphere. The origin of tropospheric ozone should, therefore, be known. The traditional theory, which considers ozone as an inert gas can only be sustained if current laboratory knowledge of photochemical reactions cannot be applied to the atmosphere and if injection of ozone through the tropopause in the NH is at least three times larger than in the SH. The determination of the origin of tropospheric ozone is fundamental in obtaining a clear picture of atmospheric chemistry and we believe that a comparison between ozone distributions in the NH and SH can serve as a useful tool in establishing the stratospheric and *in situ* tropospheric photochemical contributions. If significant tropospheric ozone production takes place, it follows that the concentrations of ozone in the lower troposphere of the NH have increased substantially since the inception of the industrial era. We need, therefore, much more meteorological and chemical (O_3 and NO) data from the SH, where ozone concentrations are certainly much closer to pre-industrial conditions because of lower technological sources of CO and NO_x .

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Model of the fine-grain component of martian soil based on Viking lander data

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The value of the sorbational specific surface of the martian soil (from CO₂ evolution in GEX (gas exchange experiments) of Viking craft) is more than an order of magnitude greater than the value of its geometrical specific surface (from granulometry). An hypothesis is therefore proposed here to explain the microporous structure of the soil grains. Absence of O₂ and CO₂ in GCMS (gas chromatography-mass spectrometry) (heating up to 500 °C) gives some indication of the closeness of the pores. The origin of such soil structure and the filling of pores with CO₂ and O₂ due to the effects of various forms of radiation are discussed. The similarity between kinetics in LR (labelled release) and GEX as well as their correspondence with the filtration curve for water vapour migrating through the soil sample suggest that both the formation and the diffusion of the gases are rapid processes. Displacement desorption by water vapour by simultaneous opening of the pores due to the Rebinder effect, is suggested as the natural mechanism for outgassing in the GEX and LR 'Viking' experiments.

A MODEL of the fine-grain component of the martian soil is proposed here. This model, based on well-known physical phenomena, enables us, in principle, to explain the mechanism of the evolution of the gases revealed in the GEX and GCMS experiments of the Viking landers¹⁻⁷. Our model proposes that the martian fines is a substance in which each grain is permeated with micropores of radius between several units and several tens of angstroms (Å), and of length ~1,000 Å. Such micropores may arise from a transfer of dissolved gas molecules from the grain bulk into lattice defects. These defects may be tracks of heavy charged particles as well as the usual structural imperfections such as dislocations. It has been shown that even mesopores can be created by bombardment with heavy multicharged ions⁸.

Formation of gases

In a substance containing a considerable percentage of oxides (such as Fe₂O₃, and CO₂) oxygen must be formed as a result of their radio and photodecomposition, in particular under strong UV radiation.

Photodecay of oxides in fine-grain substances often occurs at depths which are much greater than the range of UV photons. This seems to result from migration deep into the grain of electron excitations formed near the surface, similar to the process considered in ref. 9. To allow this phenomenon to be studied in future experiments, a theory of light scattering in such media has been developed¹⁰.

In this way one can consider formation of gases within the grain volume, which will then migrate over the volume until they encounter a free surface or the above-mentioned defects on which to form micropores to be filled with gas. Penetration of gas atoms (molecules) into these micropores will cause weakening of the van der Waals forces bracing the walls of the micropore, hence stretching them (like the inflation of a sealed

elastic bottle). The internal surfaces of such a micropore will be atomically pure initially¹¹, so that atomic oxygen on these surfaces is likely to become molecular. More specifically, on Mars, O₂ and possibly CO₂, initially dissolved in grains (as opposed to H changing to H₂ on the Moon) must accumulate in the micropores. Filling the 'bottles' in the grains of the lunar regolith with H₂ is associated with the presence of a permanent flux of solar protons which compensate for hydrogen leakage. Extralunar origins of the H₂ in lunar soils can now be considered established¹². Note that in radiation physics, the formation of gas bubbles and even lamination of targets under too high an irradiation dose (~10¹⁵–10¹⁷ particles cm⁻²) constitute the well known effect of blistering^{15,16}. The origination and development of macro- and micropores are common considerations in high temperature solid state physics¹⁷.

The phenomenon of photodecomposition in the bulk of a solid leading to the gas release is also well known. Thus, for example, it has been shown¹⁸ that irradiation of Pyrex glass by photons of about 4eV causes photodecomposition of CO₂ in the body of the glass; the CO diffuses out of the sample. In the case under consideration, photodecomposition of CO₂ dissolved in the grain would give rise to atomic oxygen migrating to the micropore where it transforms into O₂ in the physically adsorbed state. The chemisorption of O₂ is inhibited because of the lower martian temperatures. In particular, it follows that the chemisorption of oxygen on the martian grains, as proposed by Oyama *et al.*⁷, is unrealistic.

Outgassing

On heating the soil as described above, outgassing will occur mainly from the external surfaces of grains, that is from the gaps between the granules (hereafter called macropores). Outgassing from closed micropores will result from diffusion through a solid and will therefore be negligible. Moreover, some macropores and 'poorly sealed' micropores will close as a result of sintering. Such behaviour was observed in lunar material when its specific surface was reduced on heating^{8,19,20}. Nevertheless, other experiments¹² with slow heating of the lunar soil showed a sharp O₂ evolution immediately after melting. A reasonable interpretation is that in those samples charged with O₂, the gas is stored in pores which are not easily accessible.

Thus, outgassing observed in GCMS should be interpreted as being thermodesorption primarily from the macropores. Note also the absence of O₂ evolution in this experiment. Outgassing in the GEX can be explained by the fact that micropores are opened by the action of adsorbed water molecules (present in nutrient) as a result of the Rebinder effect^{17,21}. In other words, penetrating water causes an increase in the accessibility of the pores of the martian material. Highly polar water molecules cancel out the Van der Waals forces in the neck of the 'bottle' ('mouth' of a pore), the bottle tends to open and the H₂O molecules migrate into it where they are absorbed to replace gases present there—primarily O₂ and CO₂. This must cause an increase in the specific surface (rather, gas capacity) which was precisely what was observed for the lunar material^{19,20}. In this case, H₂ was mainly desorbed^{13,14}.

Our suggestion of a role for water vapour in the outgassing mechanism is strongly supported by the fact that the kinetics of

outgassing in the GEX and LR are similar and specific in character. In fact, the characteristic time t_0 of the yield of O_2 and CO_2 in both experiments is of the order of several hours. The shapes of the kinetic curves are also similar (first they rise steeply as $t^{1/4}$ and then rise exponentially to saturation). Time dependences of this kind for the total yield of products are characteristic of processes with kinetics limited by a transport mechanism of the filtration type (that is, migration through a porous medium).

Indeed, the problem of water vapour isothermal filtration through a plane layer of a porous medium of thickness L (and unit area) can be formulated in Leibenson's approximation²² which is linear with respect to concentration squared $c^2(x, t)$

$$\frac{\partial c^2}{\partial t} = k \frac{\partial^2 c^2}{\partial x^2}; \quad c(x, 0) = 0; \\ c(0, t) = c_0; \quad \frac{\partial c(L, t)}{\partial x} = 0 \quad (1)$$

where k is an appropriate coefficient of filtration²².

The standard solution of this problem gives the mean square of the concentration of vapours penetrating into the medium

$$\overline{c^2(x, t)} = \frac{1}{L} \int_0^L c^2(x, t) dx \\ = c_0^2 \left[1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{\gamma_n} \exp(-\gamma_n t/t_1) \right] \quad (2)$$

where

$$\gamma_n = (2n+1)^2 \text{ and } t_1 = \frac{4L^2}{\pi^2 k}$$

Denoting the expression in brackets in equation (2) by $\theta(t/t_1)$, we can write for the root mean squared (r.m.s.) value of the total number of water molecules, that have entered the medium at the instant t ,

$$(\overline{N^2(t)})^{1/2} = N_{\max} \cdot (\theta(t/t_1))^{1/2}; \quad N_{\max} = c_0 L \quad (3)$$

An analysis of the function $\theta(t/t_1)$ on the basis of Poisson's transformation²³ shows that $\theta(t/t_1) \approx (4/\pi^{3/2})(t/t_1)^{1/2}$ when $t/t_1 \leq 0.6$, and when $t/t_1 \geq 0.6$ according to equation (2), $\theta(t/t_1)$ exponentially approaches unity. Thus, the kinetics of gas evolution into the test cell for small and relatively large values of time in the GEX and LR becomes clear.

The time trend of gas evolution in the GEX is similar in character to that of the counts in the LR, indicating that the kinetics in both cases are controlled by the transport of the same substance whatever the nature of the other rapid processes.

Kinetics

We can now show that the transport of water vapour is responsible for these features of the kinetics curves. We must therefore evaluate the sorption heat of the migrating substance. Let the major portion of soil particles^{1-7,24} be ~ 10 – $100 \mu\text{m}$ in diameter (d). Then the characteristic molecule path length l in the gaps must be at the lower boundary of the distribution (due to packing) and we put $l = 10 \mu\text{m}$. The characteristic time t_0 (which is $\approx t_1$) during which molecules randomly migrating over the gaps leave the soil layer of thickness L , can be evaluated by equating L to the value of the r.m.s. displacement $(\Delta^2)^{1/2}$ of a molecule: $(\Delta^2)^{1/2} = L$. It is well known²⁵ that $\Delta^2 = \frac{1}{2}nl^2$, where n is the number of random steps, each having length l . It therefore seems that $n = 2(L/l)^2$. While migrating, the molecules move n times from wall to wall, stopping at each wall (becoming adsorbed) for the time τ . Therefore, neglecting the net time

of transit between the walls, we can write $t_0 = n\tau$ and, hence, $\tau = \frac{1}{2}t_0(l/L)^2$. Finally, using the elementary Frenkel relationship²⁶ $\tau = \tau_0 \exp(Q/RT)$ for the sorption heat Q we find:

$$Q = RT \ln \left[\frac{1}{2} \frac{t_0}{\tau_0} \left(\frac{l}{L} \right)^2 \right] \quad (4)$$

In our case, at $\tau_0 = 10^{-12}$ s, $l = 10^{-3}$ cm, $L = 0.5$ cm $t_0 = 3$ h = 1.08×10^4 s and $T = 280$ K then $Q = 13.3$ kcal mol⁻¹.

The value $Q = 13.3$ kcal mol⁻¹ agrees remarkably well with the value $Q_p = 13.3 \pm 0.5$ kcal mol⁻¹ for the sorption heat of water vapour on limonite, that can be readily measured from the dependences of Q_p on the water vapour pressure, given by Pollack *et al.*²⁷, equating p to the saturation pressure 7.75 torr at $T = 280$ K. Thus, the calculated value $Q = 13.3$ kcal mol⁻¹ can be definitely identified with the water sorption heat.

The fact that the sorption heat values for O_2 and CO_2 ($Q_{O_2} = 3.7$ kcal mol⁻¹ and $Q_{CO_2} = 7.3$ kcal mol⁻¹), are much smaller is due to the energetic factor causing sorption of water by replacement, that is the system goes into a lower energy state with O_2 and CO_2 being replaced by H_2O . In this case the relative amounts of gases (with respect to H_2O) in the sorbed state will be equal to the ratio of the relative surface coverages θ_i ($i = O_2$ or CO_2) to the corresponding value of θ_{H_2O} , and, according to De Boer²⁶

$$\theta_i = \theta_{H_2O} \frac{p_i}{p_{H_2O}} \exp \left(- \frac{Q_{H_2O} - Q_i}{RT} \right) = \theta_{H_2O} K_i \quad (5)$$

where p_i and p_{H_2O} are the corresponding partial pressures. Assuming that they are equal, we find $K_{CO_2} = 2 \times 10^{-5}$ and $K_{O_2} = 3.5 \times 10^{-8}$. In other words, with the penetration of H_2O , complete desorption of O_2 and CO_2 occurs. For the evolution of N_2 and Ar in the GEX, it would have been roughly consistent with their abundances in the atmosphere if their sorption heats had been equal. Perhaps, the question arising in this context can be accounted for by inability to distinguish CO from Ar (refs 1–7) (and possibly N_2).

On the other hand, it follows from equation (4) that at the indicated sorption heat values, the times $t_0 = t_{O_2}$ and $t_0 = t_{CO_2}$ of the diffusion of O_2 and CO_2 through the system of pores involved, will be $t_{O_2} \ll t_{CO_2} \ll 1$ s at $T \geq 280$ K; hence, transport of evolved gases does not contribute to the kinetics. In particular, the equilibrium concentration (or θ_i) of gases sorbed on macropores in the GCMS will instantly follow the temperature which obviously changes within time periods much longer than 1 s.

Next, compare the probable gas content in macropores and micropores of the martian soil. The gas capacity ν_μ of microporous sorbents is usually defined as the number of gas moles per gram of sorbent. The analogous quantity ν_s for macroporous dispersed sorbents with the specific surface S can be defined as $\nu_s = S/\omega N_A$, where ω is the molecule area and N_A is the Avogadro number. Evaluating S by the formula $S = 6/(\rho d)$ for $10 \mu\text{m} \leq d \leq 100 \mu\text{m}$ we obtain the gas capacity $290 \leq \nu_s \leq 2,900$ nmol g⁻¹. Estimating the relative surface coverage θ_i from the experimental sorption isotherm²⁸ for CO_2 at $p_{CO_2} = 7.3$ mbar and $T = 280$ K gives $\theta_{CO_2} \leq 0.08$. Hence, for a specimen with mass $m = 0.5$ g, the total amount of gas stored on the external surface of soil grains (that is, in macropores) will be within the range $12 < \nu_s m \theta_{CO_2} < 120$ nmol. Note that capillary condensation cannot occur at such a low value of $\theta_{CO_2} \leq 0.08$ and, therefore, this is expected to be a precise estimate.

As a result of sintering, only some of this gas will be evolved on heating, that is in the GCMS, which has been observed qualitatively¹⁻⁶. At the same time, the calculated value of $\nu_s m \theta_{CO_2}$ is 0.25–2.5% of that observed in the GEX, and, since there are no reasons to believe that $d \ll 1 \mu\text{m}$, it should be again assumed that CO_2 is mainly stored in micropores, as is all the O_2 .

The direct Viking soil measurements have shown²⁴ the diameter range to be 10–100 μm . Since the very small particles

cimen with mass $m = 0.5$ g, the total amount of gas stored on the external surface of soil grains (that is, in macropores) will (refs 29, 30) confirms the sense in our choice of diameter range.

Note that the diffusion of CO_2 from the bulk into micropores in the course of their filling can be promoted by electron excitations due to UV irradiation—similar to the migration of defects discussed in ref. 9. Hence, our assumption that the microporous structure of the fine-grain component of the martian soil has a gas capacity (in micropores) of about 10^4 mol g^{-1} reasonably explains the GEX, GCMS, and LR experiments of Viking landers. The suggested gas capacity is not large and is two or three orders lower than the gas capacity (in micropores) of such sorbents as natural coals and synthetic zeolites. For this reason and because of the natural ways of forming micropores our model is not exotic, in contrast to the widespread 'superoxide type hypothesis' which is hardly consistent with the following two experimental facts. First, absence of O_2 -evolution when heating the soil up to 500°C in GCMS, and second, absence of counts in LR after 'sterilisation' (at 160°C) ref. 3. The latter seems to give an upper limit for a characteristic decomposition temperature of the superoxide which obviously contradicts the former. Our concept also agrees quite well with the suggestion that martian soils have never been exposed to liquid water³¹.

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Muscular fatigue investigated by phosphorus nuclear magnetic resonance

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Muscular fatigue has been studied using ^{31}P NMR to measure the levels and rates of utilisation of several key metabolites and the free-energy change for ATP hydrolysis. Force development is closely correlated with metabolite levels and is proportional to the rate at which ATP is hydrolysed.

THE substances most directly involved in the transduction of chemical free energy into mechanical work in contracting muscle are ATP, ADP, orthophosphate (P_i), hydrogen ion and magnesium ion; these substances actually take part in the actomyosin ATPase reactions. Phosphocreatine (PCr) is also involved in normal contractions of vertebrate muscle, because the ATP that is hydrolysed is rapidly rephosphorylated at the expense of PCr by the enzyme creatine phosphotransferase. Recent advances in the application of phosphorus nuclear magnetic resonance (^{31}P NMR) to living muscle^{1,2} have now made it possible to monitor all these substances simultaneously, directly or indirectly, and to relate changes in them to concurrent changes in the mechanical performance of muscles.

The mechanical output of skeletal muscle declines after a sufficiently prolonged and intense period of exercise, and this decline, or fatigue, has long been a subject for experiment. As early as 1807 Berzelius believed "that the amount of free lactic acid in a muscle is proportional to the extent to which it has been previously exercised" (quoted in ref. 3). We now know a good deal more about the importance of anaerobic processes (such as lactic acid formation) in brief intense exercise, and of

oxidative metabolism in more prolonged exercise. However, there is no universal agreement about the fundamental mechanism of fatigue.

In the present article we investigate the problem of fatigue in anaerobic frog muscles undergoing repeated isometric contractions at 4°C . We ask what are the relationships between the decline in force development and the changes in all of the following: (1) concentrations of phosphorus metabolites; (2) hydrogen ion concentration; (3) the free-energy change for the hydrolysis of ATP, (which can now be estimated *in vivo* for the first time); (4) the rate of lactic acid production and (5) the rate of phosphorus utilisation. The results may permit testing *in vivo* of schemes to explain the mechanical activity of muscle in terms of the biochemistry of the actomyosin ATPase pathway.

Our experimental methods are similar to those we have developed for application of ^{31}P NMR to the study of living, contracting muscle¹. The main distinction between our experiments and more conventional NMR techniques is that the sample tube was adapted as an experimental chamber in which the muscles were mounted and superfused with physiological salt solution. The muscles were stimulated electrically via suitably placed platinum electrodes, and their tension development was measured by a silicon strain gauge.

The use of amphibian muscle at 4°C was dictated by the need to maintain the muscles in good physiological condition throughout the experiment; pairs of frog (*Rana temporaria*) gastrocnemius muscles were used because they can be large enough (300–400 mg each, wet weight) to yield an adequate signal-to-noise ratio in an experiment on a single pair of muscles. To make valid comparison between force development and the chemical environment within the muscle fibres it

is essential that every part of the muscle cross-section be in the same metabolic state. This cannot be achieved with thick active muscles if it is necessary to depend on diffusion of oxygen from the muscle surface to sustain oxidative metabolism. We therefore produced uniformly anoxic conditions throughout the muscle by gassing the circulating Ringer's solution with O_2 -free nitrogen. We found that a considerable amount of oxygen diffused through the thin plastic tubing used to convey the Ringer's solution to the spectrometer, so to ensure complete elimination of oxidative metabolism 2mM NaCN was added. Experiments using an oxygen electrode showed that this treatment eliminated oxidative metabolism within 45 min. Muscles poisoned with NaCN could be maintained at rest within the spectrometer for 12 h and more with only slight decline in their PCr content. Since we were anxious to establish whether force development is indeed a function of metabolite levels, we used three different patterns of repeated activity: stimulating for 1 s every 20 s, 1 s every 60 s, or 5 s every 300 s. We performed two experiments of each type; the total duration of the experiments varied from 18 to 92 min.

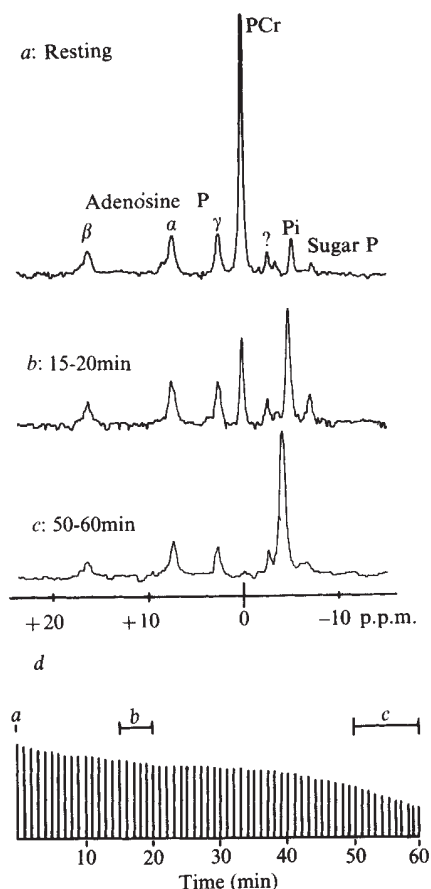


Fig. 1 Results from a single experiment in which a pair of anaerobic frog gastrocnemius muscles (total weight, 773 mg) were stimulated for 1 s every 60 s. *a, b, c*, Examples of the ^{31}P NMR spectra obtained, with peaks identified in (*a*). The multiple peak centred at -3.0 p.p.m. marked ? represents three compounds of unknown function. Two of these have been identified as glycerophosphoryl choline³³ and serine-ethanolamine phosphodiester³⁴. The peak labelled Sugar P at -7.5 p.p.m. represents combined resonances from several hexose and triose phosphates; AMP and IMP appear in the same general region¹³. We found no consistent or substantial changes in the ? or Sugar P peaks, so they are neglected in the body of the text. Spectrum (*a*) was accumulated for 10 min before the first stimulation and (*c*) is the sum of two spectra accumulated in separate 5 min bins. *d* Is a drawing indicating the average value of each force response; the initial force was 238 mN mm^{-2} .

Figure 1 shows typical spectra obtained in a single experiment in which the muscle was stimulated for 1 s every 60 s. Spectrum *a* was obtained after 2 h of anaerobiosis and immediately before stimulation. Succeeding spectra (Fig. 1*b, c*) were averaged over 5 min periods during the course of stimulation—these show a progressive decline in PCr and increase in P_i levels together with a shift of the P_i resonance in the acid direction. These metabolic changes are accompanied by a marked decline in isometric force development (Fig. 1*d*).

While spectra such as those shown in Fig. 1 yield some valuable information directly, a complete quantitative analysis of the concentrations of all of the chemical substances of interest demands a combination of NMR information with results derived from more conventional chemical techniques. Since our conclusions are dependent on the validity of this approach, which we use here for the first time, we shall describe in detail how our estimates are obtained.

The concentration of hydrogen ions was determined from the position of the P_i peak, as previously described¹. The relative amounts of ATP, PCr and P_i were obtained from measurement of peak areas which were then corrected by the appropriate saturation factors (see ref. 1, pages 709 and 710). The total phosphorus estimated in this way remained constant throughout these experiments; this provides a valuable check on the accuracy of the saturation factors used. In order to convert corrected peak areas to actual concentrations in mmol kg^{-1} it has been assumed that the resting muscle in oxygen contained 27 mmol kg^{-1} of PCr, a value derived from studies of frog sartorius muscles¹.

The [ADP] relevant to our purpose is that in free solution in contact with the myofibrils. Most of the total ADP is bound, much of it to actin where it gives no observable NMR signal⁴. The free ADP would give a signal in the region of the α and γ ATP peaks (see Fig. 1) but is present at too low a concentration to be detected, let alone measured, in the NMR spectrum from muscle. We have therefore calculated the [ADP] from its equilibrium with ATP, PCr, creatine (Cr) and H^+ , catalysed by creatine phosphotransferase. There is good experimental evidence that this equilibrium is indeed maintained in contracting muscles⁵.



$$[ADP] = [ATP] \times [Cr] / K [PCr] \times [H^+] \quad (2)$$

The equation can be written in this form because H^+ is taken up with approximately unit stoichiometry over the pH range from about 6 up to highly alkaline values⁶. Creatine cannot be determined by ^{31}P NMR. However, it is known⁵ that in fresh resting muscle the ratio $[PCr]/([PCr] + [Cr])$ is between 0.85 and 0.88 and that the Cr liberated during activity remains within the muscle^{7,8}. It is thus possible to estimate Cr from the observed changes in PCr.

Reaction (1) is strongly influenced by the free $[Mg^{2+}]$: a reworking of the results of Noda *et al.*⁹ shows that K in equation (2) remains reasonably constant at $2 \times 10^9 \text{ M}^{-1}$ if $[Mg^{2+}] > 1.5 \text{ mM}$. Our justification for using equation (2) rests partly on recent ^{31}P NMR studies on PCr¹⁰ which indicate that in resting frog muscle $[Mg^{2+}] \approx 3 \text{ mM}$, assuming that the binding constant between PCr and Mg^{2+} is 40 M^{-1} . We have looked into the question of the amount of free Mg^{2+} in frog muscle, using a method independent of any estimates of binding constants. We have examined the changes in the chemical shift for P_i and β ATP as the muscles became more acid, and compared them with the chemical shifts observed *in vitro* in mixtures of ATP (5 mM), P_i (5 mM), KCl (180 mM), $MgCl_2$ (5.1–8 mM) at varying pH. We conclude that, at least at the beginning of our experiments, $[Mg^{2+}] > 2.5 \text{ mM}$. The value of the binding constant between ATP^{4-} and $ATPMg^{2-}$ is uncertain^{11,12}, but even a low estimate¹¹ would ensure that 95% of the ATP is complexed with Mg, as reported in earlier NMR studies^{1,13–15}.

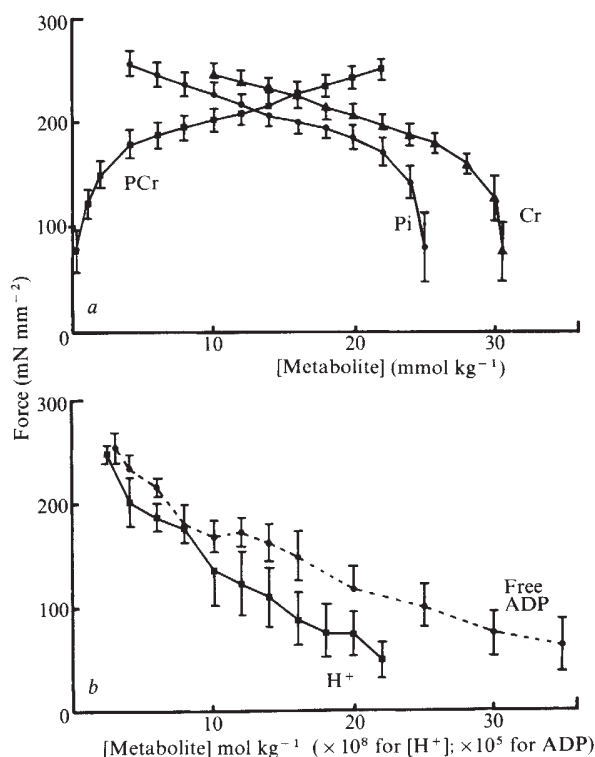


Fig. 2 Force developed as a function of metabolite levels; averaged results of six experiments. Spectra were accumulated for 5 min periods if the muscles were stimulated for 1 s every 60 s or 5 s every 5 min, and for 2 min periods if the stimulation was for 1 s every 20 s. In the 5 s/5 min experiments the average force developed by each contraction was related to the metabolite levels indicated by the spectrum preceding that contraction. For the 1-s stimulation experiments the force developed by the contraction nearest the middle of the spectral-averaging period was related to the metabolite levels. The force developed at particular metabolite levels was obtained by linear interpolation between the data points, so as to obtain the standard errors shown by vertical bars.

Experimental results

Using the methods described above we have obtained enough information to relate isometric force development in any experiment to any of the changes in concentration of substances most directly concerned with the production of mechanical activity. The different patterns of stimulation used in these experiments ensured that there were differences in all of the following: (1) the total duration of the experiment; (2) the total number of contractions; and (3) the total period for which the muscles were stimulated (that is the total number of contractions $\times$ duration of each stimulation). When force development in individual experiments was plotted against these three parameters, the results separated themselves into groups according to the different patterns of stimulation. In contrast, the relationship between force development and concentration of each of the measured substances is completely independent of the pattern of stimulation. We conclude that the decline in isometric force development is linked to the metabolic state of the muscle rather than to any independent changes in the activation of contraction. Changes in any of the processes involved in activation could be responsible for the decline in isometric force only if they themselves are somehow linked to biochemical changes; such a mechanism for fatigue has previously been suggested^{16,17}.

As there was no discernible effect of the pattern of stimulation on the biochemical results we have averaged all six experiments after grouping them for statistical purposes by linear interpolation. The results are presented so as to illustrate

the relationship between isometric force development and (1) the changes in concentration of substrate and products of the ATPase reaction, (2) the decrease in free energy available for contraction, or (3) the rate of glycolysis and the rate of cross-bridge cycling.

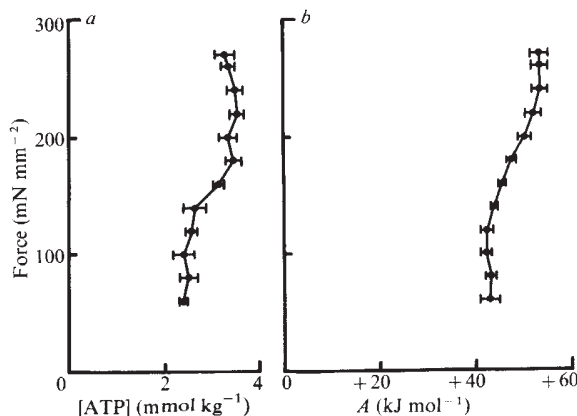
Substrate and product levels

Isometric force development is shown as a function of the changes in metabolite concentrations in Fig. 2a and b and Fig. 3a. Spande and Schottelius reported¹⁸ that in repetitively stimulated mouse soleus muscle, isometric force development is directly proportional to PCr content; they concluded that depletion of PCr is the cause of mechanical fatigue. It is clear from Fig. 2a that in our experiments isometric force development is not proportional to [PCr]; the demonstration that there is not an obligatory proportionality between these two parameters renders the above conclusion doubtful. There are, in fact, no biochemical grounds for postulating that changes in [PCr] are directly responsible for mechanical fatigue, since the primary role of PCr in muscle is to buffer the ATP and ADP levels. Figure 3a shows that [ATP] does not change until force development has declined to 60% of its initial level and then it decreases only slightly. The lost ATP is presumably converted mainly to IMP (see legend to Fig. 1). We conclude that force development is not related in a simple way to levels of substrate available for contraction, whether measured by [PCr] or [ATP].

One interesting feature of the ATP spectra is that during severe fatigue the γ and especially the β peaks shift significantly to the left. This is what would occur if the increase in [H⁺] (Fig. 2b) displaced a small quantity of Mg²⁺ from MgATP²⁻. This could be of physiological significance (since only MgATP acts as a substrate to actomyosin ATPase) and we are investigating the question further.

The decrease in force development is approximately proportional to the rise in [ADP] and in [H⁺]. Although it is too early to say whether either relation is causal rather than coincidental, a dependence of isometric force development on [ADP], [H⁺] or [Pi] could arise from product inhibition of the contractile system or from interference with activation of contraction. Force development in muscle is believed to result from cyclic attachment and detachment of myosin crossbridges to and from active sites on actin filaments. The energy required for crossbridge cycling is derived from ATP hydrolysis, the actomyosin ATPase being both structurally and functionally an integral part of this unit. It has been shown that in myosin solution (a model for resting muscle) the later stages of ATP hydrolysis are reversed by high concentrations of product¹⁹. If

Fig. 3 Force as a function of [ATP] (a) and affinity (b). The data were obtained as explained in the legend to Fig. 2. The horizontal bars represent standard errors. In (b), affinity, $\mathcal{A} = -dG/d\xi$ (kJ mol⁻¹).



a similar effect occurs with actomyosin (presumably corresponding to contracting muscle), changes in product concentrations could well affect crossbridge cycling and therefore force development. There is also evidence that $[H^+]$ may affect various stages in the activation of contraction; this has recently been discussed by Fabiato and Fabiato²⁰.

Free-energy change

It is also appropriate to relate force development to the energy available for mechanical work output. This is the free-energy change, or rather the affinity ($\mathcal{A} = -dG/d\xi$ (ref. 21)) for the hydrolysis of ATP. The affinity is equal to the maximum work theoretically obtainable per mol of ATP hydrolysis, and is conveniently calculated by the method devised by Alberty¹¹.

Maximum work per mol

$$\mathcal{A} = -(\Delta G_{\text{obs}}^0 + RT \ln [ADP] \times [P_i] / [ATP]) \quad (3)$$

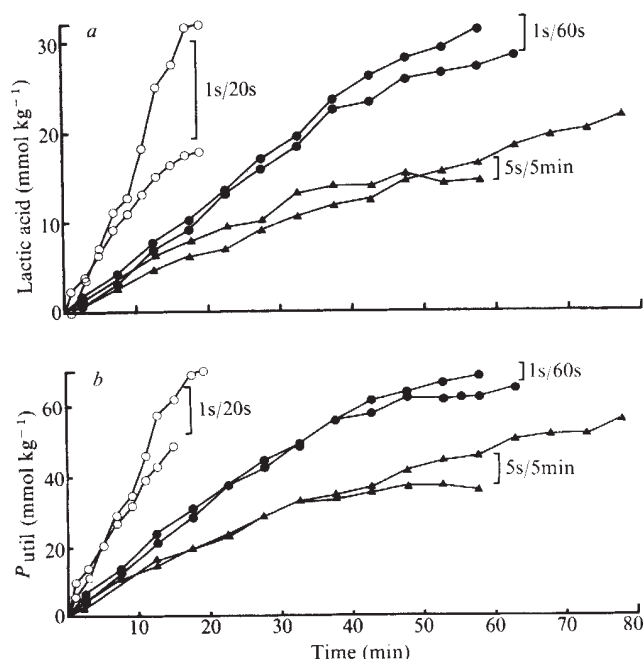
where the concentrations in square brackets are those determined analytically and include all ionised and complexed species. The effects of varying pH and $[Mg]$ are accommodated in appropriate variations of ΔG_{obs}^0 which are shown by Alberty in two-dimensional maps at various temperatures.

The affinity for the hydrolysis of ATP in these experiments can be determined from the measured metabolite concentrations by putting together equations (2) and (3):

$$\mathcal{A} = -(\Delta G_{\text{obs}}^0 + RT \ln [Cr] \times [P_i] / K[PCr] [H^+]) \quad (4)$$

The changes in isometric force are shown as a function of changes in affinity for ATP hydrolysis in Fig. 3b. These results do not relate to changes in mechanical work output (since only a very little internal mechanical work is done in isometric contractions), but reflect the relation between force development and the driving force for crossbridge cycling. It is clear from Fig. 3b that the affinity declines only slightly during fatigue and can hardly be the cause of it, unless there is a threshold value at about 40 kJ mol^{-1} below which crossbridge cycling becomes impossible.

Fig. 4 Total lactic acid produced (a) and total phosphorus utilised (b) as a function of time for each of the six experiments.



Glycolysis and ATP turnover

We have so far been concerned with relating force development to levels of affinity or of metabolites. However, the situation in muscle is dynamic, the metabolite levels reflecting the balance between the rate at which ATP is being hydrolysed and the rate at which it is being resynthesised. Comparison of the changes in rates of these reactions with the decline in force development yields additional information concerning the characteristics of fatigue.

In anaerobic conditions resynthesis of ATP occurs mainly from glycolysis, and from the breakdown of PCr. the quantity of lactic acid (LA) produced can be estimated from the pH changes and the buffering capacity of frog muscle. Other reactions known to be occurring in contracting muscle have much smaller effects on $[H^+]$ and have therefore been neglected. It is known that LA diffuses only very slowly out of frog muscle; the results of Mainwood *et al.*²² on fatigued frog muscle indicate maximum rates of LA diffusion into the medium of only a few $\text{mmol kg}^{-1} \text{h}^{-1}$. We have accordingly neglected LA diffusion from the muscle in the following calculations.

Over the relevant range of pH (6.5–7.5) the concentrations of undissociated LA ($pK = 3.9$) and of H^+ are both negligible compared with the concentrations ($>1 \text{ mM}$) that concern us here. LA^- production can thus be estimated from the increase in the BH^+ forms of the buffers present. For one buffer (B, dissociation constant K) whose total concentration ($[BH^+] + [B] = C$)

$$[BH^+] = C[H^+] / (K + [H^+]) \quad (5)$$

In muscle the production of LA^- can therefore be calculated from:

$$\Delta[LA^-] = \Delta \left\{ \frac{[\text{protein Hist}][H^+]}{K_{\text{hist}} + [H^+]} + \frac{[\text{carnosine}][H^+]}{K_{\text{carn}} + [H^+]} + \frac{[P_i][H^+]}{K_{P_i} + [H^+]} \right\} \quad (6)$$

$$= \Delta \left\{ \frac{36 \times 10^{-3}[H^+]}{3.162 \times 10^{-7} + [H^+]} + \frac{14 \times 10^{-3}[H^+]}{5.011 \times 10^{-8} + [H^+]} + \frac{[P_i][H^+]}{1.995 \times 10^{-7} + [H^+]} \right\} \quad (7)$$

The concentrations of the fixed buffers in frog muscle and all of the equilibrium constants used in this calculation are those given by Curtin and Woledge²³; $[H^+]$ and $[P_i]$ are measured as explained above. We have neglected the $\text{CO}_2/\text{bicarbonate}$ system since it was shown by Stella²⁴ that if the surrounding medium does not contain CO_2 the intracellular [bicarbonate] is only about 1 mM . Note that equations (6) and (7) take full account of the effects of PCr hydrolysis because neither PCr nor Cr has a pK near to $pH = 7$.

Total LA^- produced and total phosphorus utilised (P_{util} ; the amount of ATP breakdown that would have been required in the absence of all recovery processes) are shown in Fig. 4 as a function of time for each of the six experiments. P_{util} was calculated according to the following equation:

$$\Delta P_{\text{util}} = 1.5 \Delta[LA^-] - \Delta[PCr] - \Delta[ATP] \quad (8)$$

(There is some doubt as to the amount of ATP resynthesised per mol of LA^- produced²⁵, however, until this question is resolved we think it best to use the commonly accepted stoichiometry). Both LA^- production and phosphate utilisation depend strongly on the pattern of stimulation. Even in those cases in which the muscles were stimulated for the same total amount of time per hour (5 s/5 min and 1 s/60 s) there is a twofold difference in the total phosphorus utilised and in the total LA^- produced.

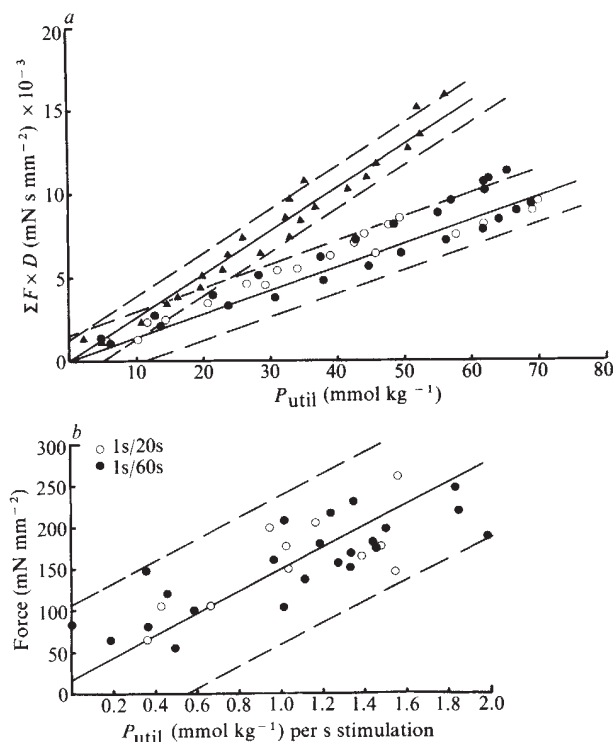


Fig. 5 *a*, The sum of the force developed in each contraction $\times$ the duration of stimulation ($\Sigma(FD)$) as a function of phosphorus utilised (P_{util}). FD was obtained by multiplying the force developed at the midpoint of each stimulation by the duration of stimulation. This method was chosen both for its simplicity and because it does not assume that ATP is being hydrolysed during the relaxation period. Δ , 5 s every 5 min; $\bullet$, 1 s every 60 s and $\circ$, 1 s every 20 s. The solid lines were obtained by least-square regression analysis, assuming that all the experimental error is associated with P_{util} . In both cases the intercepts were negligible. For 5-s stimulation ΣFD ($\text{mN mm}^{-2} \text{ s}$) = $261 P_{\text{util}}$ (mM kg^{-1}); $r = 0.99$; $n = 25$. For 1-s stimulation ΣFD ($\text{mN mm}^{-2} \text{ s}$) = $142 P_{\text{util}}$ (mmol kg^{-1}); $r = 0.97$; $n = 49$. The dashed lines are 95% confidence limits. *b*, Force as a function of P_{util} per s of stimulation, $\Delta P_{\text{util}}/D$, estimated directly from the slopes in Fig. 4*b*. For clarity only the data from stimulations of 1 s duration are included. For the 1s/20s experiments, the results were averaged over 4 rather than the usual 2 min intervals. The solid line was obtained by least-square regression analysis, assuming that all the experimental error is in P_{util} ; slope = $134 \text{ mN mm}^{-2} \text{ mmol}^{-1} \text{ kg s}$; $r = 0.80$; $n = 34$; $P < 0.001$. The dashed lines are 95% confidence limits and the intercept is not significant.

In order to relate our experimental results to theoretical schemes of contractility, we have examined the relation between force development and phosphorus utilisation in several different ways. In Fig. 5*a*, P_{util} is plotted against the sum of the force development in each contraction times the duration of stimulation ($\Sigma(FD)$). There is a highly linear relation between these two parameters; the results fall into two groups according to whether $D = 1$ s or 5 s. For each duration of contraction the results can be represented by the equation:

$$P_{\text{util}} = \Sigma FD/k \quad (9)$$

where the slope k depends on the duration of contraction.

In order to relate force developed in a single contraction to the associated chemical change, it is merely necessary to consider the increments (FD and ΔP_{util}) in both axes of Fig. 5*a* that result from an additional contraction cycle:

$$FD = k \Delta P_{\text{util}} \text{ or } F = k \Delta P_{\text{util}}/D \quad (10)$$

The latter relationship is illustrated directly for 1s contractions in Fig. 5*b*, where the values of ΔP_{util} have been taken from point-to-point gradients in Fig. 4*b*. The points closest to the

origin in Fig. 5*b* represent the end rather than the beginning of the experiment as in Fig. 5*a*. The direct interpretation of equation (10) is thus that as the muscle fatigues, the decline in force and the decline in rate of phosphorus utilisation are proportional to one another and the constant of proportionality (k) is considerably greater for the 5-s contractions ($k \approx 260$) than for 1-s contractions for which $k \approx 140$ (as determined from Fig. 5*a* or *b*).

The increased economy of contraction observed in the contractions of longer duration agrees qualitatively and quantitatively with evidence derived from studies of non-fatigued muscle. It has long been known that in single isometric tetani the rate of heat production declines after the first second of stimulation^{26,27}; a similar pattern has been demonstrated for PCr hydrolysis^{28,29}. Maréchal and Mommaerts²⁸ results on single and repeated tetani of frog sartorii at 0 °C fitted the equation

$$\Delta \text{PCr} = 0.33 + 0.28D \text{ mmol kg}^{-1} \quad (11)$$

for values of D up to 60 s. In absolute terms equation (11) accords reasonably well with other measurements on frog sartorii by chemical methods at 0 °C^{5,29,30} or by ³¹P NMR at 4 °C (ref. 1).

Our present results for $D = 1$ s and 5 s can be analysed in a similar way:

$$P_{\text{util}} = F(4.01 + 3.03 D) \times 10^{-3} \text{ mmol kg}^{-1} \quad (12)$$

This equation, and the ratio between its constants, closely resembles equation (11), and furnishes the additional information that both terms in the bracket are directly proportional to force development as it declines during fatigue. We cannot distinguish in the present experiments between phosphorus utilisation during contraction and any that is utilised between contractions but we have reason from other experiments for thinking that the latter contribution is small. Studies of non-fatigued frog (ref. 28 p. 62) and toad¹ gastrocnemius muscles at 4 °C support the view that these muscles break down 1.5–1.7 mmol kg⁻¹ of PCr during a 1-s contraction; this is in quantitative agreement with the present results and is roughly three times more than the values obtained on sartorius muscles at 0–4 °C.

In trying to relate our results to the fundamental processes thought to be occurring in contracting muscle, equations (11) and (12) are particularly attractive since it seems natural to associate the first bracketed term with the chemical and mechanical events required to set up isometric force; while the second, duration-dependent term could be associated with the actual crossbridge cycling that maintains the force.

Certainly there is good evidence that a quantity of PCr is hydrolysed at the onset of contraction even when actomyosin interaction has been prevented by stretching^{31,32}. If the duration-dependent term in equation (12) can indeed be equated with crossbridge cycling, then our results show that the economy of maintenance of force remains constant in fatiguing muscle at about 300 N mm⁻² s per mol ATP kg⁻¹ split, and that the force declines because the rate of ATP utilisation falls progressively. If so, anything that reduced the rate of ATP hydrolysis (for example product inhibition or decreased Ca²⁺ release) would automatically reduce the force proportionately and result in fatigue.

Our results show that force development is closely correlated with metabolite levels rather than with any independent changes in excitatory conduction. We also find that the decline in isometric force development as the muscle fatigues is proportional to the decrease in rate of phosphorus utilisation; the latter result suggests that the hydrolysis of ATP in each crossbridge cycle produces a fixed mechanical impulse (force $\times$ time) and that the economy of ATP hydrolysis remains unchanged.

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Transformation by concanavalin A of the response of molluscan neurones to L-glutamate

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Concanavalin A causes in all Aplysia and Helix neurones a depolarising response to L-glutamate. The Con A-induced glutamate response is pharmacologically distinct from the three cell-specific glutamate responses (two inhibitory, one excitatory) that can be elicited from untreated molluscan neurones. The transformation in glutamate sensitivity brought about by Con A does not seem to be related to the lectin's capacity to produce redistribution of receptor sites in cell membranes.

THE plant lectin concanavalin A (Con A) binds to specific polysaccharide residues of membrane glycoproteins¹. The effect of Con A on neurotransmitter receptors is of interest because many of these receptors are thought to be glycoproteins. However, electrophysiological studies of lectin action are rare, and effects on transmitter responses have been observed in only one preparation. Mathers and Usherwood² demonstrated that the response of the locust muscle to an iontophoretic application of the presumed neuromuscular transmitter, L-glutamate, was altered by incubation of the preparation in Con A. I report here a study of the effects of this lectin on the sensitivity of molluscan neurones to the same amino acid.

In molluscan neurones, three different types of response to L-glutamate can be observed^{3–8}. Two types of inhibition can be seen; one due to a selective increase in Cl[−] permeability, the other to a selective increase in K⁺ permeability. An excitatory response can also be observed, and seems to be due, at least in part, to the movement of Na⁺. As will be shown below, Con A alters the membranes of molluscan neurones in such a way that an additional excitatory response to L-glutamate develops on all cells, accompanying and often masking the original cell-specific effects of this amino acid. The effect of Con A observed here does not seem to depend on a redistribution of the lectin's binding sites.

Transformation of glutamate sensitivity by Con A

As the Con A-induced effect described here was found to occur on all cells of both *Aplysia* and *Helix* ganglia, independent of

their normal response to L-glutamate, the conclusions reported here have been simplified by using only data obtained from the medial cells⁹ of the pleural ganglion of *Aplysia californica* (for the single exception, see Fig. 3a).

A brief pulse of L-glutamate applied to the somatic membrane of a medial cell, exposed by removal of the overlying connective tissue, elicits an inhibitory response that inverts at the equilibrium potential for Cl[−] ions (Fig. 1, control). Following a 40-min exposure to bath-applied Con A, this same cell is depolarised and excited by an identical pulse of glutamate (Fig. 1, Con A). The Con A-induced depolarising response to L-glutamate develops gradually in all of the exposed cells of the ganglion. In the medial cells, the net potential change eventually becomes excitatory, although the increase in Cl[−] permeability can still be detected, often by a shortlived break in the burst of action potentials triggered by the glutamate application (see bottom series of records in Fig. 1).

The amplitude of the Con A-induced glutamate response increases with Con A concentration (up to approximately 500 µg ml^{−1}) and with the time of exposure to the lectin (up to at least one hour). With a dose of 100 µg ml^{−1}, a change in glutamate sensitivity can be detected as early as 30 s after the solution is changed. The same type of transformation as that shown in Fig. 1 can be obtained with concentrations as low as 10 µg ml^{−1} (Fig. 4, Con A); however, the evolution of the response is slower and the amplitude of the final Con A-induced glutamate response is smaller. If, for example, a preparation is exposed to Con A for only 5, 10 or 15 min, the Con A-induced glutamate response remains at the amplitude measured at the end of a given exposure to the lectin, and does not progress further after the exchange of the Con A-containing seawater for normal seawater. The lectin-induced response remains for at least 24 h (the maximum time tested) after washing out the Con A-containing seawater.

Con A has been shown to be displaced from its binding sites by O-methyl-α-D-mannopyranoside (αMM) (see ref. 10 for recent evaluation). By competing with the membrane polysaccharide Con A binding sites, this sugar reverses many Con A-induced membrane changes. When the Con A-treated *Aplysia* ganglion was bathed in seawater containing αMM (10^{−2} g ml^{−1}) the Con A-induced glutamate response was eliminated. This finding, shown in Fig. 2, indicates that the effect of Con A requires the continued presence of the lectin on the

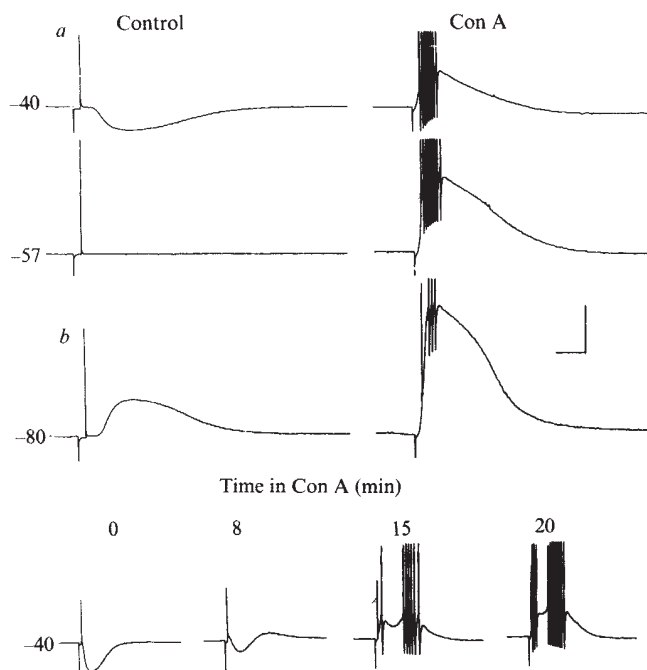


Fig. 1 Effect of Con A on the response of medial cells to L-glutamate. *a*, Control, selective increase in Cl^- permeability ($E_{\text{Cl}} = 57 \text{ mV}$) elicited in a normal medial cell by an iontophoretic application of L-glutamate (10^{-3} M , pH 8) from a $150 \text{ M}\Omega$ electrode. Con A, response of the same cell to the same pulse of L-glutamate following a 40-min exposure to $500 \mu\text{g ml}^{-1}$ Con A. Note that after Con A incubation the net response to L-glutamate is excitatory rather than inhibitory. *b*, Evolution of the Con A-induced transformation in the glutamate response of a medial cell as a function of increasing exposure (in min) to Con A ($200 \mu\text{g ml}^{-1}$). Note that the normal Cl^- -dependent inhibition remains visible as a break in the train of action potentials resulting from the Con A-induced element of the glutamate response. Calibration, 2 s, 20 mV (upper end of action potential traces cut off). In all experiments, double-barrelled microelectrodes filled with $0.5 \text{ M K}_2\text{SO}_4$ were used for recording responses and for controlling membrane potential. The resistance of the glutamate electrodes varied over $60\text{--}100 \text{ M}\Omega$ for the pressure pipettes, and over $100\text{--}250 \text{ M}\Omega$ for the iontophoretic pipettes.

membrane, and that the change in sensitivity to glutamate is not the result of a transformation triggered by, but no longer dependent on, the Con A binding.

Sodium dependence of the Con A-induced glutamate response

The Con A-induced glutamate response is still depolarising at $+10 \text{ mV}$. Because of the marked delayed rectification seen over the more positive potential range, the inversion potential of the response could not be reached. This limitation made it difficult to evaluate the exact contribution of changes in permeability to different ions. It can be stated, however, that the glutamate response which appears after Con A incubation does not depend on the presence of Ca^{2+} in the seawater, as it does not decrease in amplitude when the normal seawater is replaced by Ca^{2+} -free seawater. In contrast, the response completely disappears when the NaCl of the seawater is replaced by either Tris-Cl or by sucrose. The response returns when Na^+ -containing seawater is reintroduced.

Pharmacological specificity of the Con A-induced response

To evaluate the specificity of the Con A-induced membrane change, a series of compounds shown to imitate L-glutamate in other preparations^{4,7,8,11-14} was tested on *Aplysia* neurones.

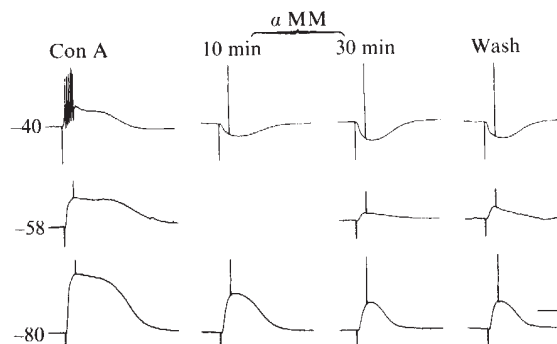
Among the compounds tested were D-glutamate, DL-ibotenate and quisqualate. Each of these compounds, the effectiveness of which was compared with that of L-glutamate by the use of double-barrelled iontophoretic electrodes, was found capable of imitating at least one of the normal glutamate response types. Ibotenate elicits only the Cl^- -dependent response (that is, the type seen in the medial cells), quisqualate is most effective in eliciting the K^+ -dependent inhibition, such as that seen in certain buccal neurones, whereas D-glutamate was found to be an effective activator of the normally occurring Na^+ -dependent response.

The inhibitory responses elicited by ibotenate and quisqualate were found to be qualitatively unchanged by exposure of the ganglion to the lectin. For example, the response of the medial cells to ibotenate continued to be a pure Cl^- -dependent inhibition, even after a long exposure to Con A. Similarly, the K^+ -dependent response of certain buccal neurones to quisqualate remained unaffected by the lectin. The response of these same cells to L-glutamate, in contrast, was markedly transformed, with the development of a depolarising element that readily dominated and eventually masked the normally occurring hyperpolarising responses.

To study the effect of Con A on the normally occurring excitatory response, which in *Aplysia* neurones can be obtained only from restricted regions of the axonal membrane, the depolarisations elicited by the D- and L-isomers were compared before and after exposure of the ganglion to the Con A-containing seawater. When equal doses of the two isomers are applied, the response to D-glutamate is always the smaller of the two, although it often reaches half the amplitude of the response to the L-isomer (for example, Fig. 3*a*, control). Whether elicited by D- or by L-glutamate, these highly localised depolarising responses increase when the ganglion is exposed to Con A (Fig. 3*a*, Con A).

Although the D-isomer can imitate the cell-specific, highly localised excitatory action of L-glutamate seen in normal cells, it cannot activate the Con A-induced response elicited by L-glutamate over the entire exposed membrane of all cells, irrespective of their normal response to glutamate (Fig. 3*b*). The marked stereospecificity of the Con A-induced glutamate response suggests that this new, generalised response is not simply the result of a spreading, over the entire cell surface, of the non-stereospecific, axonally localised receptors which

Fig. 2 Elimination of the Con A-induced change in glutamate sensitivity obtained with exposure of the ganglion to α -D-methylmannopyranoside (αMM). In a medial cell exposed for 30 min to Con A ($200 \mu\text{g ml}^{-1}$) the response to a pressure application of L-glutamate (10^{-2} M L-glutamate, pH 8) was changed from the normal Cl^- -dependent inhibition (no records shown) to a net excitatory response (see Con A). After washing away the Con A-containing seawater, the Con A records were taken, and then the ganglion was bathed in seawater containing αMM ($10^{-2} \text{ g ml}^{-1}$) (see 10-min and 30-min exposures). αMM reversed the Con A effect, causing the L-glutamate response to become inhibitory once more. This return to a normal glutamate sensitivity persisted even after washing away the sugar-containing seawater (see Wash). Calibration 5 s, 20 mV.



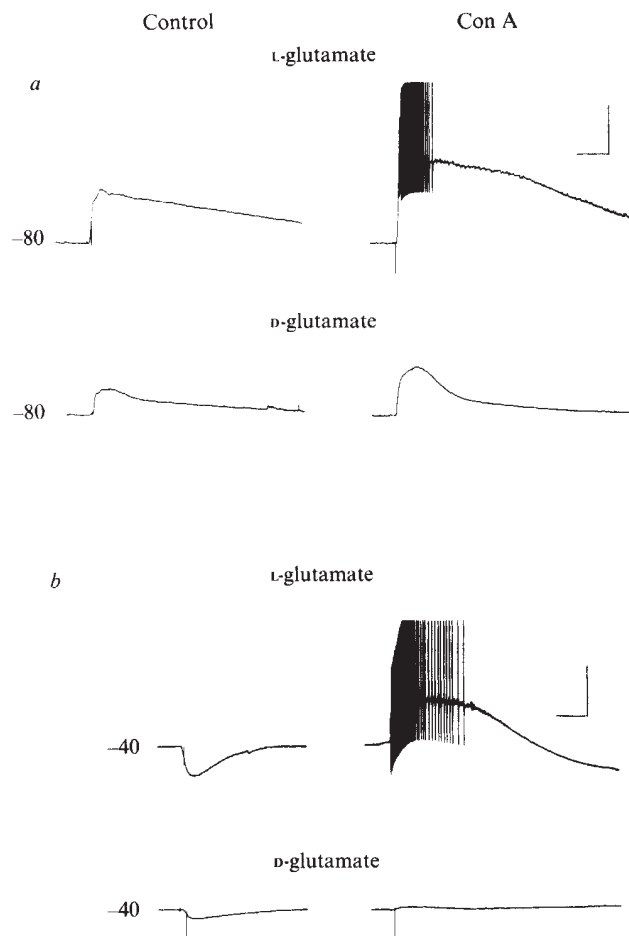


Fig. 3 Differential effect of Con A on the axonal response of a buccal cell (*a*) and the somatic response of a medial cell (*b*) to pressure applications of L- and D-glutamate. *a*, Control, the normal depolarising response of a buccal cell to D-glutamate is about half the amplitude of its response to L-glutamate (each pipette filled with a 10^{-1} M concentration). Con A, note that whereas the response of D-glutamate was moderately increased after a 45-min exposure to Con A ($500 \mu\text{g ml}^{-1}$), that to L-glutamate was markedly increased. *b*, Control, the Cl^- -dependent response of a normal medial cell to a pressure application of 1 M D-glutamate is much smaller than that to a pressure application of a 10-fold lower concentration of L-glutamate. Con A, after exposure for 1 h to $500 \mu\text{g ml}^{-1}$ Con A, L-glutamate elicits a marked depolarisation, whereas a 10-fold higher concentration of D-glutamate elicits only a 1 mV depolarisation (L-glutamate pipette, 10^{-1} M; D-glutamate pipette, 1 M). Calibration 2 s, 20 mV.

mediate the Na^+ -dependent response of the normal cells. Rather, a pharmacologically distinct receptor seems to have been revealed by the lectin.

As mentioned above, Con A causes an increase in the normally occurring excitatory response, whether it be elicited by D- or by L-glutamate. However, the increase in the L-glutamate response is usually much greater than that of the D-glutamate response. This added increase in the response to L-glutamate is most probably due to a superposition of the 'new', stereospecific response on the Con A-facilitated, D-glutamate-sensitive, normally occurring response.

Relation of the Con A effect on molluscan neurones to the ability of the lectin to control receptor movements in other systems

Con A has been shown to have a wide variety of biological effects, most of which involve control over lateral movements

of membrane receptors. For example, the binding of Con A to many membranes results in a marked redistribution of the lectin's own binding sites¹⁵⁻²¹. In contrast, Con A has also been shown to inhibit the movement of receptors for other ligands; for example, it inhibits the movement of anti-immunoglobulin receptors when bound by their antibody²¹⁻²³.

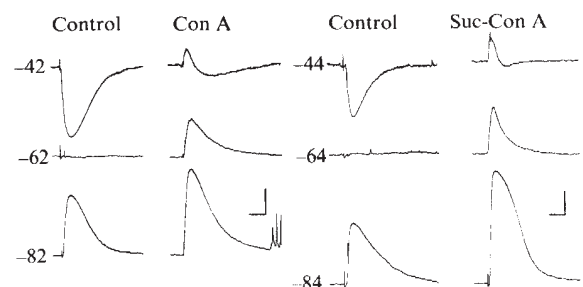
Ligand-induced redistribution of receptors in membranes usually involves an initial local regrouping of the randomly distributed binding sites into so-called patches or clusters. This action of Con A depends on three variables. First, the capacity of Con A to control receptor movement depends on the temperature at which incubation in Con A takes place. At low temperatures, Con A continues to bind (albeit often less effectively^{24,25}). However, the sites to which it binds do not move into clusters until several minutes after the preparation has been warmed^{15,16,18,19}. Second, integrity of the microtubules is apparently important for this Con A effect^{23,26-30}, as treatment of animal cells with colchicine blocks or reverses the lectin's control over receptor movement. Finally, the ability of Con A to displace its own binding sites^{19,31} or to control displacement of receptors for other ligands^{21,26,32} is lost when the Con A molecule, normally a tetramer, is broken down into dimers by succinylation. The change in effectiveness of the succinylated Con A cannot be attributed to an alteration in its binding capacity, which remains normal³². These three variables were manipulated to see if the Con A effect on molluscan neurones might be related to redistribution of receptor sites in the neuronal membrane.

To test the effect of temperature on the Con A-induced transformation of glutamate sensitivity on *Aplysia* neurones, the ganglion was cooled to 0°C , incubated for 40 min with Con A at the same temperature, then washed at 0°C . Just before testing the glutamate response, the ganglion was rinsed with 20°C seawater. The glutamate response was measured no later than 1 min after the initial rise in temperature. Following such a procedure the response of a medial neurone to glutamate was found to be comparable with that observed in a medial cell of another ganglion which had been exposed to Con A at ambient temperature.

Likewise, when the ganglion was incubated with the microtubule-disrupting drug colchicine (10^{-3} g ml^{-1}), the treatment neither impeded the development of the new glutamate response, nor eliminated the response if it had previously been established. Thus, microtubular integrity does not seem to be relevant to the Con A-induced transformation observed in molluscan neurones.

Finally, when *Aplysia* ganglia were incubated in seawater containing succinyl-Con A (that is, the dimeric form of the lectin), the transformation of membrane sensitivity to L-glutamate was the same as that induced by tetrameric Con A at equivalent concentrations (mg ml^{-1}). Figure 4 gives a

Fig. 4 Comparison of the effect of the native, tetrameric Con A (Con A) and its succinylated, dimeric form (Suc-Con A) on the L-glutamate responses of two medial cells. Both experiments were carried out on medial cells, but of necessity cells from different pleural ganglia. As can be seen by comparing the two sets of records, equivalent exposures (40 min) to equivalent concentrations ($10 \mu\text{g ml}^{-1}$) yielded a similar transformation in the response of each medial cell to equivalent applications (by pressure) of L-glutamate. Calibration, 2 s, 5 mV.



comparison of the responses obtained with the two forms of Con A at $10 \mu\text{g ml}^{-1}$.

These data all strongly suggest that one of the most thoroughly studied and ubiquitous actions of lectins, the control over lateral movement of membrane receptors, is not relevant to the effect of Con A observed in molluscan neurones.

Discussion

The pharmacological analysis of the responses described here suggests that there are normally three different types of glutamate receptor present in the normal molluscan nervous system, and that following incubation with Con A, a fourth, pharmacologically distinct, receptor is revealed.

The only previously published electrophysiological observation of a Con A-induced change in neurotransmitter responses is that made in locust muscle². Normally, there is a decrease in responsiveness of this muscle to repeated iontophoretic applications of its presumed transmitter, L-glutamate. Mathers and Usherwood² found that this characteristic response decrement ('desensitisation') disappears after exposure of the muscle to Con A.

It is very difficult to think of the major transformation in glutamate sensitivity that is seen in all molluscan neurones in terms of a change in desensitisation. Unlike the case described in the locust, the Con A-induced glutamate response observed in *Aplysia* appears where no glutamate depolarisations can be detected before Con A incubation. On the other hand, further study might reveal a closer parallel between the Con A-induced 'change in desensitisation' of the locust muscle and the Con A-induced 'facilitation' (Fig. 3a) of the 'normal' excitatory response in molluscan neurones.

No data are yet available for determining which of the many possible sites of action of Con A might be involved in these transformations. However, the capacity of Con A to alter the glutamate sensitivity of molluscan neurones does not appear to

depend on its ability to control the lateral movement of membrane receptors.

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Sensory neurones recognise defined pathways in *Drosophila* central nervous system

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An analysis of the central projection of various sensory neurones in the homoeotic mutant bithorax postbithorax, and in flies where an adult nerve had been experimentally misrouted, reveals that neurones are able to develop a normal projection even if they enter the central nervous system at an unusual place.

THE function of the nervous system depends on the development of specific connections between nerve cells. The mechanism by which neurites find their way to their synaptic targets is not known. It has been shown that regenerating axons are able to reestablish appropriate connections with a high accuracy, both centrally and peripherally (see refs in ref. 1). However, the mechanism which leads to specific reinnervation may be different from the mechanism which allows the normal development of connectivity in the nervous system; for example, regenerating fibres may use clues laid down by the original neurites². It would therefore be interesting to follow the fate of identified neurones which develop at an ectopic location, and to analyse the paths followed by their neurites.

In insects, sensory neurones are derived from the epidermis, have their cell bodies peripherally beneath the sensory structures, and send their axons centripetally towards the central

nervous system. In the holometabolous *Drosophila*, the bulk of the adult sensory axons develop at the time the imaginal disks differentiate, early during metamorphosis. Homoeotic mutations are known in *Drosophila* which modify the state of determination of imaginal disks. It has been shown in the case of mutations which transform the antenna into a leg that ectopic leg chemosensory neurones establish functional connections similar to those of homologous neurones on the normal legs^{3,4}. The paths followed by the axons of these cells have not been determined.

Here, I analyse the paths followed by various axons forced to enter the central nervous system at an abnormal place as a result of genetic or surgical interference with normal development, and compare them with the normal paths of these axons.

Normal and homoeotic projections from the thoracic bristles

The relevant parts of the peripheral nervous system of *Drosophila* are shown diagrammatically in Fig. 1. In normal flies (Fig. 1, left) the metathorax is reduced to a narrow strip of cuticle which bears the halteres. The double mutation *bithorax postbithorax* (*bx pbx*, Fig. 1 right) transforms the metathorax into a mesothorax⁵. In the genetic combinations used in this work

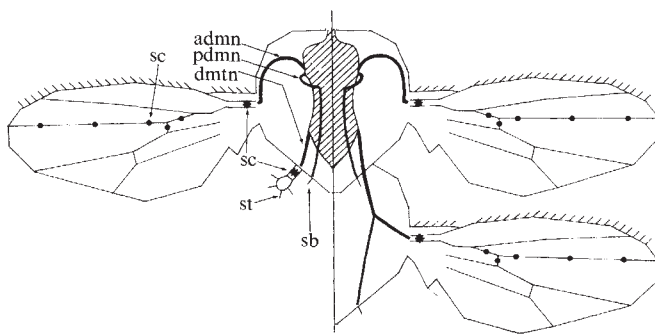


Fig. 1 Diagram of the relevant sensory structures on the thorax of a normal (left) and *bithorax postbithorax* (right) *Drosophila*. Large dots represent single sensilla campaniformia (sc) on the wing blade; asterisks represent groups of these sensilla at the base of the wing and on the stalk of the haltere; short bars represent sensory bristles on the wing margin, sensilla trichoidea (st) on the halter knob and the anterior scutellar bristles (sb) at the posterior edge of the notum. The sensory bristles which cover most of the notum have not been represented; their axons join those from the scutellar neurones. Heavy lines show schematically the peripheral path of the corresponding axons. The axons from the wing sensory structures form the anterior dorsal mesothoracic nerve (admn), the axons from the bristles on the notum form the posterior dorsal mesothoracic nerve (pdmn), and the axons from the haltere (left) or from the homoeotic notum and wing (right) form the dorsal metathoracic nerve (dmtn). The shaded area represents the thoracoabdominal ganglion.

(see legend to Fig. 2), the homoeotic mesothorax and wings are almost as large as the normal structures, and bear the full complement of bristles and sensilla found on the normal mesothorax. The nerves from the homoeotic structures follow the path of normal metathoracic nerves and enter the thoracic ganglion at a place far posterior to the entry of the normal mesothoracic nerves. This provides a situation in which the neurones underlying given sensory structures may be observed to enter the central nervous system either at a normal place, if the sensory cells belong to the normal mesothorax, or at a different place, if these cells belong to the homoeotic mesothorax.

Two pairs of large bristles, the scutellars, are found at the posterior edge of the notum, the dorsal region of the mesothorax. The sensory neurones underlying either of these bristles have been backfilled with horseradish peroxidase. The same pathway is followed by both axons in wild type as well as in *bx pbx* flies; this is shown in Fig. 2a in the case of the left anterior scutellar bristle of the normal notum of a *bx pbx* fly. The projection of the neurone underlying the left anterior scutellar bristle of the homoeotic notum of a mutant fly is shown in Fig. 2b. The axon enters the ganglion at a place which corresponds to the dorsal metathoracic nerve, comes to a point close to the normal path and then bifurcates. Both branches turn abruptly, one in an anterior direction, the other in a posterior direction. The anterior branch follows fairly exactly, though in the opposite direction, the anterior path of the pathway followed by the normal axon, including the contralateral branch and the laterally ramified branches. The second branch follows the remaining part of the normal path.

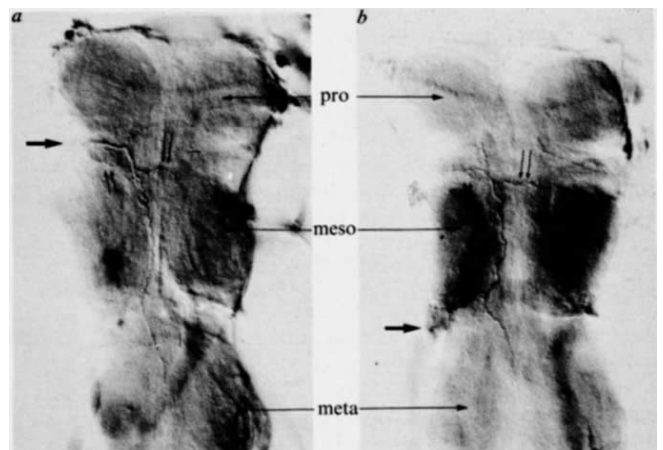
The central projection of the small bristles (microchaetes) which cover most of the notum has also been investigated. It was observed that the sensory neurones which underlie the microchaetes of the central region of the notum, between the anterior suture and the scutellar groove, all share a common projection (Fig. 3a, left). Therefore, the observations were usually made on groups of 10–15 of these bristles. The neurones underlying the large bristles (macrochaetes) which develop in the same region are difficult to backfill; the few successful cases reveal a projection similar to that of the microchaetes (Fig. 3a, right). In *bx pbx* flies, the neurones of the normal microchaetes develop a normal projection (Fig. 3b,

right). On the other hand, the axons corresponding to the microchaetes on the homoeotic notum enter the ganglion more posteriorly, move in an anterior direction and then reproduce the normal projection (Fig. 3b, left). The fibres which cross the midline are contributed by both the normal and the homoeotic axons, as can be observed in preparations in which either of them are backfilled.

The normal projections from the wing and haltere

The central projection of the wing sensory neurones is rather complex (Fig. 4A, dorsal and 4B, ventral). Furthermore, part of this projection seems to be homologous with part of the haltere projection, which would complicate the comparison of normal and homoeotic wing projections. Thus, it was found necessary to 'dissect' these projections to identify suitable sensory neurones. This was done by cutting the wing at different places so that the axons from different sensory structures could be specifically backfilled.

Fig. 2 Path of the axon from the left anterior scutellar bristle of the normal (a) and of the homoeotic (b) notum of a *bx pbx* fly. The bristles were removed from their sockets and the underlying nerve cells were backfilled with a solution of horseradish peroxidase (Sigma type VI, 50 mg ml⁻¹ 0.1 M KCl). The thorax was cut open in formaldehyde-phosphate (pH 7.6) fixative, fixed for 30–60 min, rinsed for 30 min in sucrose (8%), incubated for 30 min in diaminobenzidine tetrahydrochloride (1 mg ml⁻¹ Tris 0.12 M, pH 7.6). H₂O₂ was added (0.02% final) and the reaction was allowed to develop for 5–45 min. (This method is modified from ref. 12 and G. Toubreau, personal communication.) The preparation was then dehydrated and the ganglion was dissected out, cleared and mounted. All the figures of homoeotic backfills represent flies of the genotype *bx³ pbx/Ubx¹⁰⁵*. This genotype shows complete penetrance, high expressivity and good viability. However, all the results reported here were also observed in flies heterozygous for either *bx³* or *bx³ pbx* on one chromosome, and *Df(3)P9, Ubx⁷⁵* or *Ubx¹⁰⁵* on the other chromosome. Abbreviations: meso, mesothoracic neuropile; meta, metathoracic neuropile; pro, prothoracic neuropile; heavy arrow indicates the place of entry of the backfilled axons in the ganglion; double arrows indicate specific features of the projection (see text). Nomarski optics, magnification × 350.



All the sensory bristles on the anterior margin of the wing, including those on the costa, give rise to a dense projection between the pro- and mesothoracic neuropiles (Fig. 4B, a). All the sensilla campaniformia on the wing, except the group at the base of the radius, are responsible for the projections b and c of Fig. 4B. In particular, the five sensilla along the third vein and the anterior crossvein send at least three fibres along path b and at least one along path c. The projections d, e and f of Fig. 4A correspond to a large group of sensilla campaniformia at

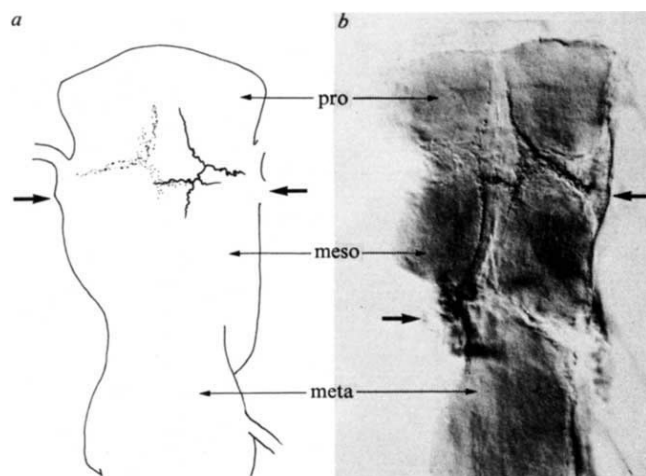


Fig. 3 Path of the axons from dorsocentral bristles. *a*, wild-type fly: 15 microchaetes were removed from the central region of the left half of the notum; the anterior dorsocentral macrochaete was removed from the right half of the notum. The dotted appearance is typical of the projection from the microchaetes; very fine fibres can sometimes be distinguished among the dots. Camera lucida drawing, X350. *b*, *bx pbx* fly: 15 microchaetes were removed from the right half of the normal notum and 15 from the left half of the homoeotic notum. The procedure was as in Fig. 1 except that the time allowed for backfill was 6 h. Microchaetes were removed from their sockets with fine tweezers or by scraping with the tip of a glass pipette. Abbreviations as in Fig. 1. Nomarski optics, magnification $\times 350$.

the base of the radius, and possibly to the chordotonal organs of the wing base. The projection which corresponds to the bristles of the tegula, a sclerite at the wing base, has not been represented; it resembles the projection of the dorsocentral microchaetes except that there is no contralateral branch.

The minute bristles (sensilla trichoidea) which are found on the haltere knob (capitellum) project as a bundle between the meso- and metathoracic neuropiles (Fig. 4C, *a*). The bulk of the haltere projection derives from the haltere stalk (pedicel and scabellum). It is a huge tract of many fibres going to the brain, with a bush of short branches directed medioposteriorly, at the root of the haltere nerve, and some lateral branching at the root of the mesothoracic nerves (Fig. 4C, *b*). This projection corresponds to the numerous sensilla campaniformia which are arranged in rows on the haltere stalk. In individuals where the ipsilateral wing and haltere projections are filled, this projection extensively overlaps the projections *d* and *e* of the wing. Finally, the projection *c* of Fig. 4C, which has no correspondent in the wing projection, arises from sensory structures at the very base of the haltere, possibly the chordotonal organs.

It is interesting that similar sense organs found on developmentally homologous⁶ regions of the normal wing and haltere should establish homologous projections in the central nervous system. For example, the bristles on the wing margin and the sensilla trichoidea on the haltere knob send similar short, dense projections ventromedially, one anterior to the mesothoracic and the other anterior to the metathoracic neuropile. Likewise, the sensilla campaniformia on the base of the wing and on the developmentally homologous stalk of the halteres seem to recognise the same path which extends from the metathoracic neuropile to the brain. These preliminary results may indicate that the establishment of sensory projections in the central nervous system depends on the state of a developmental programme much as the differentiation of the external morphology of the adult does⁷. The use of morphogenetic mosaics^{6,8} should allow a detailed analysis of this possibility.

Normal and homoeotic projections from the distal sensilla of the wing

The description of the wing and haltere projections indicate that the distal sensilla campaniformia of the wing are best suited for a comparison of normal and homoeotic situations. Indeed, they have no metathoracic counterpart, nor does any haltere sensory structure project along part of the same path. The sensilla along the third vein and anterior crossvein send their axons along that vein towards the central nervous system, and are therefore easy to backfill independently of any other wing sensory structure. The axons of the most distal four of these sensilla were traced in normal and homoeotic flies.

The projection from these sensilla for normal flies is shown in Fig. 4B, *b* and *c*, but the same pattern is observed after backfilling the sensilla of the normal wing of a mutant fly (Fig. 5, right). The projection of the sensilla of the homoeotic wing is shown in Fig. 5, left. The axons enter the ganglion posterior to the mesothoracic neuropile, but manage to reproduce the normal projection in all its details. In particular, the medialmost path is complete with its characteristic ramifications in the anterior region and its slightly dorsal, perpendicular branch at the level of the mesothoracic neuropile. The lateralmost projection shows the typical oblique anteromedial branch at the level of the mesothoracic neuropile, and the posterior coalescence with the medialmost fibres.

The projection of misrouted haltere axons

It has been shown⁹ that surgical operations on mature larvae aimed at breaking the larval nerve which connects an imaginal disk to the central nervous system can result in the misrouting of the corresponding adult nerve and its entering the thoracic ganglion at the position appropriate for another adult nerve. This technique has been used to misroute the haltere nerve and analyse the fate of the dorsalmost projection of the haltere (Fig. 4C and Fig. 6a). This projection is specific to the haltere and develops more dorsally than any other haltere or wing projection, thus making its path easy to identify.

The result (Fig. 6b) shows that a normal projection is established by haltere axons which have been misrouted through the posterior dorsal mesothoracic nerve. A delicate branch of this projection extends posteriorly along the stretch

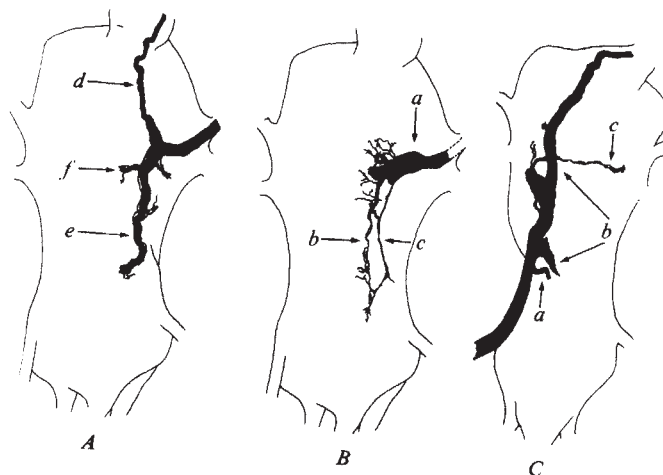


Fig. 4 Projections from the wing (*A*, dorsal component and *B*, ventral component of the complete projection) and haltere (*C*) of a wild-type fly. In *A*, *f* is more dorsal than *d* and *e*; in *B*, *a* is slightly ventral to *b* and *c*; in *C*, *a* is most ventral and *c* most dorsal. All these projections are reproducible except for the lateral branching of the haltere projection *b* which is somewhat variable and is not considered in the test. The procedure was as in Fig. 1 except that the concentration of peroxidase was $10\text{--}15\text{ mg ml}^{-1}$ and the time left for backfill was 2 h. Abbreviations as in Fig. 1, *a*–*f* refer to the paths commented on in the text.

which would have been followed anteriorly had the haltere nerve entered the ganglion at the appropriate place.

Discussion

Before analysing the results obtained with the mutant *bx pbx*, one has to consider the suitability of the use of homoeotic mutants for studying nerve projections. Homoeotic mutations in the *bithorax* locus affect the state of determination of specific imaginal disks, or of parts of them. They have also been shown to affect the larval epidermis of the corresponding segments as judged by the modifications in the pattern of cuticular outgrowths. A deficiency for this locus also affects the pattern of tracheation of the larva (E. Lewis, personal communication). The larval musculature is also modified by various mutations in this locus (Y. Jan, L. Jan and E. Lewis, in preparation). Thus, it seems possible that these mutations also affect the central nervous system, complicating the analysis of the sensory projections observed in such flies. However, it should be noted that all the effects mentioned above may be strictly dependent on an effect on the epidermis. The tracheal system develops as invaginations of the lateral embryonic ectoderm which also gives rise to the larval hypoderm and to the imaginal disks¹⁰. Furthermore, epidermis seems to be a determinant of the arrangement of the muscles, at least in the adult (I. Deak, in preparation). The results presented here give no indication that the central nervous system may be directly affected by the

Fig. 5 Projection of the four distalmost sensilla campaniformia of the normal (right) and homoeotic (left) wing blades in a *bx pbx* fly. The third vein was broken proximal to the anterior crossvein in the right normal wing and in the left homoeotic wing. The procedure was as in Fig. 4 except that the time of backfill was 3 h. a, b, c, d; 'optical sections' from dorsal to ventral, Nomarski optics, $\times 350$.

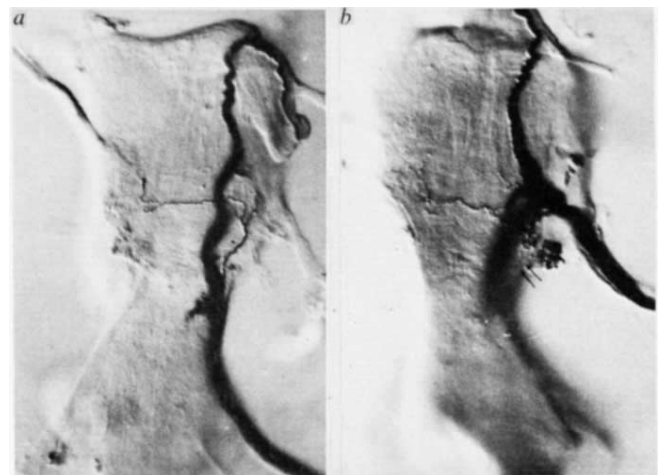
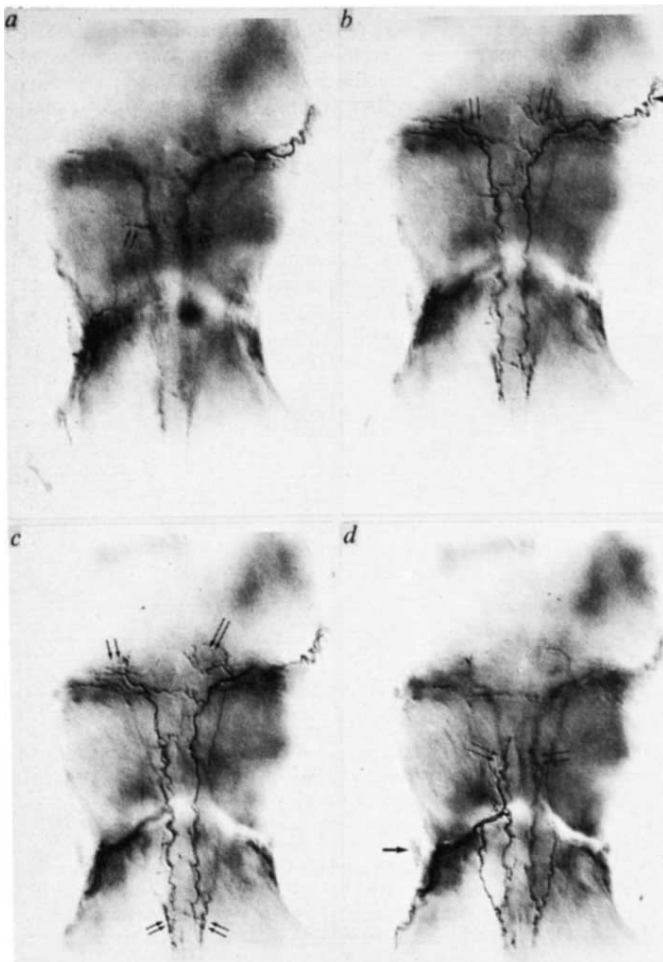


Fig. 6 The dorsalmost projection of the haltere in a wild type fly (a) and in a fly where the haltere nerve is misrouted and joins the posterior dorsal mesothoracic nerve (b). Nomarski optics, $\times 350$. The procedure was as in Fig. 4.

homoeotic mutations examined. No effect could be detected on the internal organisation of the thoracic ganglion as observed with phase and Nomarski optics, which give an excellent resolution of most of the tracts described by Power¹¹. More specifically, the projection of various mesothoracic structures is identical in normal and mutant flies. Thus, it seems that, as far as the projections studied here are concerned, the *bx pbx* mutations have no effect on the central nervous system.

The results obtained with various homoeotic sensory neurones, as well as with experimentally misrouted axons, all point to the same conclusion: a sensory axon is able to establish the appropriate projection even if it enters the ganglion at a wrong place. In many cases, given parts of a path are followed by a homoeotic axon in a direction opposite to that normally followed. In particular, a homoeotic axon which reaches its prospective projection at the middle of a straight stretch will bifurcate and follow it both ways. It should be noted that in all cases reported here, the misrouted axons entered the ganglion not too far from some part of the normal path they would eventually follow. It is possible that they would fail to establish the normal projection if this distance were too great.

These results suggest that the growing axon recognises a specific 'trail', which it follows regardless of where the first contact was made. This specification of the course to be followed may ensure that a given axon will be brought in close proximity to its synaptic target(s). It will be interesting to examine the distribution of synaptic regions along these specified paths.

I thank E. Lewis for information about, and mutations in, the *bithorax* locus, and A. Résibois for discussions. A comparison of whole projections from normal and homoeotic wing and haltere was carried out independently by J. Palka (in preparation). This work was carried out under contract between the Belgian government and the Université Libre de Bruxelles. I am supported by the Fonds National de la Recherche Scientifique (Belgium).

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letters to nature

Relation between monthly variations of global ozone and solar activity

STUDIES of Dobson measurements have revealed long-term variations of total ozone of the order of 11 yr (refs 1–5). However, there is a lack of consensus as to whether these long-term variations are in response to changes in solar activity^{5–9}. It has been suggested⁸ that shorter-term responses might be detected if a relationship between ozone and solar activity exists. In this study the shorter-term monthly averaged variations of global ozone, determined from infrared interferometer spectrometer (IRIS) measurements aboard Nimbus 4, are shown to be consistently in agreement with monthly averaged variations of solar activity. Such natural variations must be taken into account when trying to isolate the effects of anthropogenic species such as chlorofluoromethanes and nitrogen fertilisers on the ozone layer. The relationship between monthly changes of ozone and solar activity are found to be generally consistent with the longer-term ozone variations over the last solar cycle and can be accounted for by assuming monthly solar flux variations near 0.2 μm of about 2%.

IRIS data were chosen as a measure of monthly variations of global ozone because of their superior spatial coverage compared to the data from the Dobson network (less than 100 stations) or from the backscattered ultraviolet (BUV) experiment aboard Nimbus 4 (limited to measurements below 59° latitude in the winter hemisphere at solstice and only approaching the latitudinal coverage of IRIS a few weeks near equinoxes). The IRIS experiment obtained approximately 10^6 measurements of total ozone during day and night between $\pm 82^\circ$ latitude (99% of the Earth) from April 1970 to January 1971. Although IRIS was superior in spatial coverage, a prerequisite of determining accurate global mean ozone concentrations, the simplified reduction technique used to reduce the measurements could have produced systematic errors and there is evidence of inconsistencies with Dobson measurements¹⁰. Nevertheless, it was thought that relative changes associated with monthly variations of solar activity might be revealed even though absolute values might be in error.

Using an approach similar to that used earlier for species in the thermosphere¹¹, the global distribution of ozone was expressed in terms of a spherical harmonic expansion. IRIS data for each month were averaged into 1,296 cells of 5° latitude by 10° longitude and area weighted. Random noise of $\pm 6\%$ (ref. 10) was assumed for each of the $\sim 10^5$ independent ozone measurements each month. The resulting average ozone values were fitted by weighted least-mean-squares to a 9th order, 9th degree spherical harmonic expansion of the global ozone distribution¹². The first term of the 100-term spherical harmonic expansion (zero order, zero degree) is the global mean ozone. The standard deviation in the global mean ozone taking into account (assumed) random noise, but not possible bias, was generally less than 0.1 Dobson unit ($0.1 \times 10^{-3} \text{ atm-cm}$). Thus very small changes in global ozone could be detected.

Since the monthly variations of the solar ultraviolet radiation near 0.2 μm which may cause ozone variations were not measured during the IRIS experiment, a solar activity index which is measured daily from the ground was chosen for this initial study—the 10.7 cm solar flux. This index has been used

successfully to predict the large atmospheric variations in the Earth's thermosphere and exosphere associated with variations of extreme ultraviolet solar radiation¹³.

The monthly averages of the global mean ozone and of the 10.7-cm solar flux are shown in Fig. 1. Although the mean annual variations of ozone for the Northern and Southern Hemispheres were substantial, the global mean combining the two hemispheres remains nearly constant. The small monthly variations in the global mean ozone are remarkably consistent with the monthly variations of solar activity. Without exception each time there is an increase or decrease in solar activity, there was a corresponding increase or decrease of global mean ozone.

The linear least-mean-square relationship between the global ozone and solar activity is given by

$$\bar{O}_3 = (309.0 \pm 0.3) + (0.11 \pm 0.03)(\bar{F}_{10} - 154.4) \quad (1)$$

where $\bar{O}_3$ is the monthly mean global ozone (in 10^{-3} atm cm) and $\bar{F}_{10}$ is the monthly mean 10.7 cm solar flux (in $10^{-22} \text{ W m}^{-2} \text{ Hz}^{-1}$). The coefficient of correlation between $\bar{F}_{10}$ and $\bar{O}_3$ is found to be 0.75 which exceeds the 95% confidence level for a positive correlation. When the monthly time rate of change of ozone was correlated with the monthly time rate of change of $\bar{F}_{10}$ a slightly lower slope was found for equation (1) (0.09 ± 0.02) and a slightly higher coefficient of correlation was obtained (0.78).

It is of interest to compare these short-term variations with long-term variations in ozone over the last solar cycle. The yearly mean $\bar{F}_{10}$ increased from 81 to 156 between 1963 and 1970. Equation (1) would predict a 2.8% increase in ozone if the monthly relation based on data near solar maximum applied throughout the solar cycle and was the only significant factor affecting global ozone. The global increase in ozone from 1963 to 1970 (determined from Dobson data by area weighting climatic zones, see ref. 15, page 74, Fig. 5) is approximately 3%, and is therefore consistent with equation (1). After 1970 (the most recent year of maximum 10.7-cm solar flux) the ozone concentrations and $\bar{F}_{10}$ start to decrease in accord with equation (1).

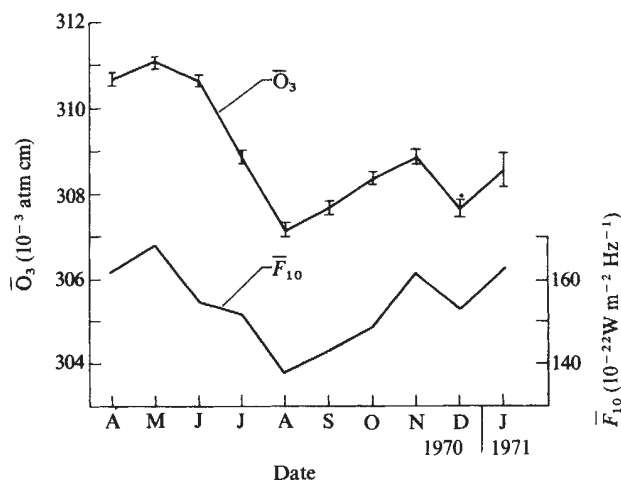


Fig. 1 Comparison of monthly-mean global ozone ($\bar{O}_3$) measurements from the Nimbus 4 IRIS experiment and monthly-mean 10.7 cm solar activity index ($\bar{F}_{10}$). Error bars, ± 2 s.d.

Finally, there is the question of what could cause the relationship between ozone and solar activity. Ruderman and Chamberlain¹⁵ have suggested that the decrease of the solar magnetic field near solar minimum modulates cosmic rays and allows an increase of low energy cosmic rays to penetrate into the atmosphere thereby increasing NO_x and destroying ozone. However, the cosmic ray variation has a lag time of about 1 yr relative to solar activity indices¹⁵ and thus the observed monthly variations in ozone which are seen in Fig. 1 to be essentially in phase with solar activity are inconsistent with such a mechanism. Although the cosmic ray variation does not seem to be the cause of the observed monthly variations, it may be a factor in the longer-term 11-yr variation. It is more likely that the monthly variations in ozone are related to monthly variations of solar ultraviolet radiation. Evidence of solar ultraviolet variability has been recently reviewed by Heath and Thekaekara¹⁶ and their results are consistent with a substantial variation in solar ultraviolet radiation near 0.2 μm over the 11-yr solar cycle with the largest variation at the shortest wave lengths. Scaling the theoretical results of ref. 17, it is estimated that the 3% variation in ozone over the last solar cycle could be produced by a $\sim 15\%$ variation in the solar ultraviolet radiation between 0.1754 and 0.2105 μm assuming no variation above 0.2105 μm . The observed monthly variations of ozone could, therefore, be due to monthly variations of solar ultraviolet radiation of less than 3%. The estimates are based on a one-dimensional radiative-convective-photochemical model and the predicted increase in ozone with increased solar ultraviolet flux takes into account both enhanced photochemical formation of ozone and photodissociation of N₂O which reduces the NO_x available for ozone destruction. Increases in the solar flux above 0.2105 μm would increase destruction of ozone and act to increase somewhat the required variation below 0.2105 μm . The atmospheric response time will also tend to increase the variation in solar ultraviolet required to match the observed monthly ozone variability. The time required for the ozone to reach an equilibrium state after a change in solar flux has been estimated as <3 months (ref. 18). This suggests that monthly changes in solar flux may generally prevent the ozone level from reaching full equilibrium.

This report constitutes the first evidence of a relationship between short-term variations of ozone and solar activity. This relationship probably results from solar ultraviolet variability near 0.2 μm .

Note added in proof: Adjusting for the theoretical variation in ozone due to variations in Earth-Sun distance (M. Natarajan, personal communication) improves the coefficient of correlation between global ozone and the $\bar{F}_{10}$ solar activity index.

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Modulational instability of whistler-mode signals

DATA from ground based and satellite observations have confirmed the occurrence of quasi-period very low-frequency (VLF) whistler-mode signals in the Earth's magnetosphere¹ and the solar wind². I report here that new regions of modulational instability could cause whistler wave modulation in the magnetosphere. I also indicate the importance of whistler modulation instability in other astrophysical and laboratory plasmas.

In both space and laboratory plasmas, whistlers could be excited by a long-wire antenna or by numerous instabilities. The latter usually occur in the presence of the stream of electron beams or an anisotropic velocity distribution in a medium. Nonlinear effects such as the particle trapping in the wave field can halt the wave growth. Hence, steady state saturation sets in and finite amplitude whistler wave-trains originate, which are ducted along the force lines of the geomagnetic field. It is widely thought that during the course of the whistler-wave propagation, nonlinear effects such as the wave-wave coupling also play a vital role. For example, interaction of a powerful VLF whistler-mode wave with ultra low-frequency (ULF) pulsations leads to the amplitude modulation of a whistler. This phenomenon is experimentally observed in space plasmas²⁻⁶ and has created considerable laboratory interest⁷. Brinca⁸ explained the magnetospheric observations in terms of the modulational instability of the whistler wave. He emphasised the importance of the trapped-particle modulational instability in which the nonlinearity introduces a frequency shift proportional to the square root of the whistler wave amplitude. Within this model, the e -folding time of the modulational instability was found to be 100 s. Because the time required for a whistler to cross the possibly modulational unstable equatorial region is ~ 0.7 s, it was concluded that modulational instability associated with trapped particle effects could not explain the amplitude modulation of the whistlers in the magnetosphere.

Recently, Karpman and Washimi⁹ obtained new regions of modulational instability in directions oblique to the initial whistler wave propagation. This could be responsible for the whistler wave modulation in the magnetosphere. The physics involved in the instability mechanism is basically the parametric excitation of waves in a plasma. According to the theory of parametric interaction, a finite amplitude whistler wave ($\omega = c^2 k^2 \Omega_e / (\omega_{pe}^2 + c^2 k^2)$, $k \parallel B_0 \hat{z}$, $\omega_{pe}^2 \gg \Omega_e^2$, $\omega < \Omega_e$; ω_{pe} and Ω_e are the electron plasma and gyrofrequencies, respectively, $k = 2\pi/\lambda$, λ is the parallel wavelength of the whistlers, and c is the velocity of light) propagating along the external magnetic field $B_0 \hat{z}$ nonlinearly couples with self-generated non-ducted ULF fast magnetosonic waves ($\Omega \approx \kappa v_A$; $\kappa = (\kappa_x, 0, \kappa_z)$, where $v_A = B_0 / (4\pi n_0 m_i)^{1/2}$ is the Alfvén speed). These waves, of course, satisfy the momentum and energy conservation relations. The sidebands and whistler pump beat together and produce a low-frequency ponderomotive force. The latter enhances the original density or magnetic field modulation. Thus, nonlinear coupling of a modulated whistler wave (represented as a quasi-particle) with a fast magnetosonic mode produces a strong resonant interaction⁹ and instability. The efficient coupling occurs when the 'Cherenkov resonance condition' $v_g \cos \theta = v_p = v_A$ is satisfied. Here, $\cos \theta = \kappa_z / \kappa_x$, v_p is the phase velocity of the fast magnetosonic modes, $v_g = \partial\omega/\partial k = 2\omega(\Omega_e - \omega)/k\Omega_e$ is the group velocity of the whistler wave-packets. The increment γ of the modulational instability is given by⁹

$$\gamma = (\omega \kappa v_A)^{1/2} \alpha H / 8^{1/2} B_0 (1 - \alpha)^{1/2} \quad (1)$$

where $\kappa = (\kappa_x^2 + \kappa_z^2)^{1/2}$, $\kappa_z \ll \kappa_x$, $\alpha = \omega/\Omega_e$, $\Omega_e = |eB_0/m_e c|$, and

H is the whistler wave magnetic field. (1) was obtained for $\gamma/\Omega = \gamma/kv_A \ll 1$ and is valid for

$$(H/B_0)^2(k/\kappa)(v_g/v_A)\alpha^2/(1-\alpha)^2 \ll 1 \quad (2)$$

For $\kappa/k \leq (m_e/m_i)^{1/2}$ this gives $H/B_0 < (m_e/m_i)^{1/2}/\alpha$.

We now apply the above results to the magnetosphere at the height $L \sim 3$ in the equatorial region. Taking the typical plasma parameters $m_i/m_e = 1,836$, $\alpha \approx 0.4$, $\omega/2\pi = 15$ kHz, $\omega_{pe}/\Omega_e \approx 7$, $\omega_{pe} = (4\pi n_0 e^2/m_e)^{1/2}$, $n_0 \approx 10^3$ cm $^{-3}$, $H/B_0 \sim 10^{-2}$, $k = 2\pi/\lambda$, $\lambda \sim 2 \times 10^5$ cm, we find that the e -folding time γ^{-1} of the modulation is roughly 0.2 s.

Hence, the amplitude modulation develops in less time than that taken by the wave transit through the equatorial region of the magnetosphere. Note that the time taken by a whistler to cross the latter is < 1 s and the ULF-modulation frequency Ω is ~ 25 Hz. Thus, our theoretical results are in excellent agreement with the experimental observations³⁻⁶ in the Earth's magnetosphere.

To show the importance of the new whistler modulational instability, we have applied our results to the magnetospheric plasma. However, our results are of great interest in the study of nonlinear whistler wave modulation in the ionosphere, solar wind², as well as in the recent laboratory experiments⁶. To support this view, we substitute the relevant plasma parameters in equation (1) and note that the e -folding time of the modulation is of the order of 0.1 s. Subsequently, the nonlinear effect, as discussed here, takes place before the whistler-mode signals escape from the plasma. We have only considered here the initial stage of the whistler modulational instability. In the long term, modulations may evolve and eventually localise. This can be considered as a nonlinear stage of the modulational instability. What one observes now is the envelope whistler solutions⁹. The latter consist of self-consistent low-frequency density depressions in which high-frequency whistler wave envelopes are trapped. Such a localised structure is actually observed in the upstream direction of the solar wind² and in a recent laboratory experiment⁷. This observation supports the important role of nonlinear wave-wave coupling phenomena not only in the magnetosphere but also in other plasmas of our general interest.

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Photocatalytic oxidation of organic compounds on Mars

THE non-detection of organic compounds on Mars¹ is interesting because there are at least two mechanisms that can produce a contemporary accumulation of these compounds on Mars—photochemical synthesis and meteoritic infall. The synthesis of organic compounds from carbon monoxide and water absorbed in inorganic matrices under UV irradiation and simulated martian conditions has been demonstrated¹. By comparison, our Moon has a surface composition that is 1.1% carbonaceous chondrite, due to a meteoritic infall rate about three times less than that on Mars². As much as 5% of carbonaceous chondrite material is organic³. Assuming a meteoritic

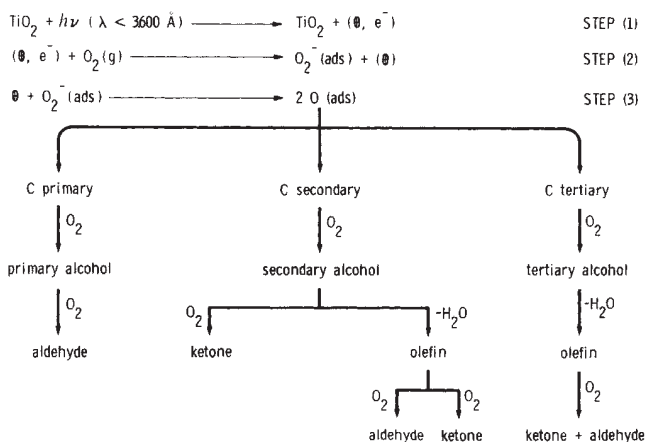
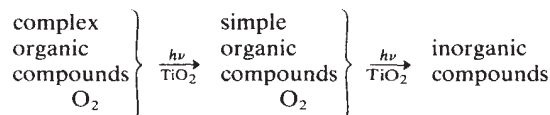


Fig. 1 Step (1): Formenti *et al.*¹⁵ suggest that with the absorption of a proper quantum, an electron-hole pair is generated in titanium dioxide. Step (2): the free electron is trapped by molecular oxygen adsorbed from gas phase forming $\text{O}_2^-(\text{ads})$ species and freeing a positive hole. Step (3): the positive hole then reacts with the $\text{O}_2^-(\text{ads})$ species forming two oxygen atoms. The oxygen atom reacts with a carbon centre to form an alcohol intermediate which can be dehydrated to yield olefin. Further oxidation of the alcohol intermediate or olefin requires lattice oxygen activated by UV irradiation, yielding an aldehyde or a ketone or inorganic molecules. The reactivity of the oxygen atom with a carbon centre in a hydrocarbon chain is related to the electron density of the carbon centre and steric effect. The following sequence of reactivities of the different types of carbon centre has also been determined. Tertiary C > quaternary C > secondary C > primary C. $\bullet$, Positive hole; C, carbon centre.

influx rate three times that of the Moon, and a reasonable mixing rate with the martian regolith, organic constituents of carbonaceous chondrites should be diluted in the martian soil to concentrations detectable by the Viking gas chromatograph-mass spectrometer (GCMS)¹. The results of the Viking GCMS experiments were, therefore, rather surprising. No organic molecules were present in the Viking GCMS samples above concentrations of parts per 10^9 (ref. 1). Mechanisms have been proposed in which peroxides, superoxides and ozonides formed under UV irradiation oxidise organic compounds^{4,5}. Glow discharge was suggested as the 'scavenger' of martian organic matter⁶. Here, we propose an alternative mechanism for the destruction of organics on Mars—UV-stimulated catalytic oxidation. Our proposal differs from previous ones in that all conditions required for photocatalytic oxidation of organics—gaseous oxygen⁷, UV irradiation⁸, and titanium dioxide^{9,10}—have been found to be present in the martian environment.

The degradation process from complex organic into inorganic compounds is:



The tendency of organic compounds to oxidise in the presence of oxygen and a catalyst, such as titanium dioxide, when irradiated with UV light has been widely reported¹¹⁻¹⁴. Formenti *et al.* suggest that under illumination by UV of the proper wavelength, an electron-hole pair is created at the surface of titanium dioxide¹⁵. Oxygen atoms, formed from O_2 , are then involved in the oxidation of alkanes. The overall reaction is summarised in Fig. 1. Bickley *et al.*¹⁴, however, suggest that hydroxyl ions on the surface of titanium dioxide are involved in the formation of oxygen atoms.

We believe that UV-stimulated oxidation is a process likely to occur on Mars because all three conditions—gaseous oxygen, UV irradiation, and a catalyst (titanium dioxide)—have

been found to be present in the martian environment. First, Mars has a 7.6-mbar atmosphere, of which 0.13% or 0.01 mbar is oxygen⁷. $O_2^-(ads)$ species (explained in step (2) of the figure legend) have been detected in photo-reactions where the partial pressure of oxygen in the ambient atmosphere is as low as 10^{-2} torr or 0.01 mbar (ref. 16). Second, the oxidation of organics also means that titanium dioxide needs to be illuminated with UV light of wavelength shorter than 3,600 Å. Although $O_2^-(ads)$ species can be photo-formed by wavelengths as long as 5,500 Å, only wavelengths shorter than 3,600 Å are capable of generating electron-hole pairs on the surface of titanium dioxide, needed to interact with $O_2^-(ads)$ species for the generation of oxygen atoms¹⁵. UV radiation was reported to reach the martian surface with little attenuation at wavelengths as short as 2,000 Å during non-dust storm conditions⁸. Observation from the Viking landers revealed atmospheric attenuation of a few tens of per cent during quiescent periods, and an order of magnitude reduction in solar flux during dust storms. The attenuation by dust and clouds was found to vary little with wavelength¹⁷. From these observations we may conclude that UV radiation with sufficient intensity and proper wavelength usually reaches the martian surface to stimulate oxidation of surface material, except during brief intervals of intense dust storm activity. Finally, the presence of titanium dioxide is another condition necessary for the process to occur. Analysis of the wavelength dependence of the absorption index of martian dust⁹ and Viking inorganic analysis¹⁰ revealed that about 1% of the martian surface material is titanium dioxide. The photocatalytic activity of other oxides have also been investigated, it was found that titanium dioxide is by far the most efficient catalyst that participates in the UV-stimulated oxidation of organics.

Low martian temperatures pose no problem for the photo-process. At temperatures as low as -50°C , formation of acetone from the photo-oxidation of isobutane on titanium dioxide in a static type reactor can be detected using infrared (IR) spectroscopy¹⁵. Although UV-stimulated catalytic oxidation is a surface reaction, complete oxidation of organic compounds in the mixed martian regolith is possible. Eolian abrasion on Mars is believed to be more efficient than on Earth. Such weathering processes can effectively remove the weathered layers and expose fresh layers of material to atmospheric oxygen and UV irradiation.

Having considered all the conditions required for UV-stimulated oxidation of organics, it seems plausible that this process is responsible for degrading organic molecules ultimately into inorganic end products. We are testing the proposed mechanism under laboratory conditions, simulated closely to the martian surface environment and the results will be reported elsewhere.

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Absorption by water vapour at 7.1 cm^{-1} and its temperature dependence

IN a study of the molecular composition of water vapour we have compared absorption measured at a wave number of about 7 cm^{-1} with that predicted for vapour consisting only of single molecules. The $7\text{--}10\text{ cm}^{-1}$ spectral region lies between monomer rotation lines where predicted absorption is low, but where anomalies in the absorption spectrum of the atmosphere require explanation¹⁻⁴. In this experiment a large excess absorption with a quadratic pressure dependence was observed and this is attributed to the presence of water dimers. However, the temperature dependence of the excess absorption at low temperatures is much stronger than that expected from equilibrium concentrations of dimers.

Measurements were made with an untuned cavity following a technique developed originally for the determination of acoustic loss in gases⁵ and later adapted for measurement of electromagnetic loss in the centimetre wavelength region⁶. In this application to short millimetre waves an important innovation is the use of a single radiation detector outside the cavity which allows the use of sensitive or complex detectors. The experimental methods will be fully described elsewhere and only a brief description is given here.

The untuned cavity or electromagnetic reverberation chamber consists of an approximately cylindrical copper box ($\sim 60\text{ cm}$ in diameter and 1 m long) acting as the absorption cell. At millimetre wavelengths these dimensions give the cavity a high density of modes and the reflectivity of copper makes the Q value large. The change in power level at the detector on introducing a lossy gas to the cell is equivalent to absorption in a path of about 40 m —the exact value can be determined by the introduction of a known loss. The cavity was excited by about 1 mW of second harmonic power at 7.1 cm^{-1} (213 GHz) from an IMPATT diode oscillator⁷ of which the fundamental was rejected by a waveguide taper. The intensity of the radiation being reverberated in the cavity is sampled by measuring the power passing through a hole in the wall by a Golay cell or helium-cooled bolometer. The effect of introducing a gas is observed and from this its absorption coefficient can be computed by expressions given by Lamb⁸. The mode density, which is already large from the size and irregular shape of the cavity, is further increased by a mechanical scrambler which rotates with a period which is short compared with the time taken for a single measurement.

To simulate atmospheric conditions, the samples were water vapour at various pressures mixed with 700 torr of nitrogen. Sample results for high and low temperatures are given in Fig. 1 which also shows the predicted absorption by water monomers calculated from the parameters described in Table 1. It is seen that the magnitude of observed absorption is

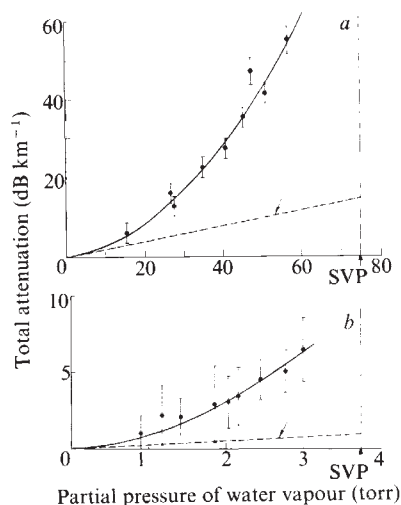


Fig. 1 The dependence of observed absorption at 7.1 cm^{-1} on partial pressure of water vapour plus 700 torr of nitrogen for two temperatures near the extremes of the range investigated. *a*, 320 K; *b*, 270 K. The experimental points are well represented by the sum of linear and quadratic terms of which the former is consistent with the monomer prediction, which is shown as the dashed line.

greater than prediction at all pressures and that the dependence is clearly not linear. The observations can be fitted by a combination of linear and quadratic components in which the linear component is attributed to the monomer and is consistent with prediction. When the predicted monomer absorption is subtracted from the observed values the quadratic pressure dependence of the excess absorption is taken as evidence for the existence of an additional absorbing species of which the concentration is itself increasing quadratically with the partial pressure of water vapour.

Water dimers, formed from pairs of water molecules joined by single hydrogen bonds and of which persuasive theoretical descriptions¹⁴⁻¹⁶ have now been given, would be expected to have an equilibrium concentration n_d which varies quadratically with monomer density n_m and exponentially with temperature as in the expression

$$n_d = \sigma n_m^2 \exp \left[\frac{E_B}{kT} \right] \quad (1)$$

where σ is a scale factor whose temperature dependence is small compared to that of the exponential factor. For dimers in equilibrium with monomers the binding energy E_B will be close to 0.16 eV per molecule which is a net theoretical value deduced from the total electronic binding energy given by Dierksen *et al.*¹⁵ and the zero point energies for the monomer and dimer derived from vibrational frequencies predicted by Curtiss and Pople¹⁶.

Figure 2 shows the quadratic component of absorption in excess of prediction derived from observations like those in Fig. 1 and taken at different temperatures. The expected behaviour of equilibrium dimers with a binding energy of 0.16 eV is shown as a dashed line which has been fitted to the high temperature observations. This does not adequately represent what is found at temperatures below about 300 K where the absorption increases more rapidly with decreasing temperature than would be expected from equilibrium dimers.

This behaviour resembles results of an investigation of the speed of sound in water vapour¹⁷ in which the sum of two exponential terms was required to describe the anomaly. One, which dominated at the higher temperatures, represented a dimer with binding energy of $0.16 \pm 0.09 \text{ eV}$, as predicted, while the other term which dominated at the lower temperatures showed a much higher binding energy of $1.4 \pm 0.3 \text{ eV}$. It is

found that the absorption anomaly, β , measured here can be described in a similar way by an expression of the form:

$$\beta = C_1 \exp \left[\frac{0.16 \text{ eV}}{kT} \right] + C_2 \exp \left[\frac{\epsilon}{kT} \right] \quad (2)$$

where C_1 , C_2 and ϵ were found by least squares analysis. The value of ϵ found was $0.9 \pm 0.5 \text{ eV}$ which is in agreement with the value found in the speed of sound determination. We believe that the results presented here constitute the first evidence for such behaviour of absorption in a laboratory experiment although measurements of absorption in the atmosphere for this wave number range had previously shown steeper temperature dependence than expected for dimers in equilibrium¹.

The high value of ϵ cannot be reconciled with a binding energy of an equilibrium state of water dimers. To identify ϵ with a binding energy is to assume equilibrium behaviour, so the physical significance of the steep temperature dependence must be questioned, and some other interpretation given to this important anomaly.

Tentatively we offer the hypothesis that the anomalous absorption below 300 K is still due to water dimers because of the quadratic dependence of its strength on partial pressure, but at these low temperatures a non-equilibrium state is present. The steep temperature dependence would then be characteristic of the processes of relaxation from that state. The presumption that a non-equilibrium condition exists then raises the question of how it is maintained. Either our experiments were all made on water vapour samples which were in that state from the initial evaporation process and had not relaxed or alternatively they were being maintained in a steady state by an input of energy. Without being categorical, we favour the second alternative as a result of experiments where delays of up to an hour were deliberately introduced between evaporation and measurement and which showed no evidence of hysteresis. Two sources of input energy to the water vapour can however be identified in our experiments. These are the millimetre wave photons being used for measurement and a small amount of acoustic power from the rotating mode scrambler. In the absence of additional evidence we cannot comment further on the effectiveness of either of these sources.

With such results it is important to consider possible experimental artefacts which might be significant. The most important of these would be neglect of the effect of adsorbed or

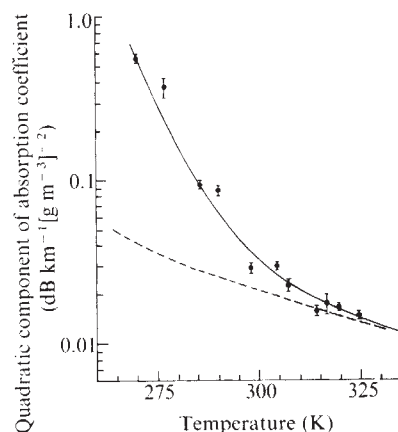


Fig. 2 The quadratic terms derived from plots represented by pressure scans such as those in Fig. 1 and given in practical units as a function of temperature. The temperature dependence of absorption due to equilibrium dimers with a binding energy of 0.16 eV per molecule, fitted at high temperatures, is shown dashed. The solid curve represents a linear combination of two exponential terms fitted to all points where one exponent is assumed to be 0.16 eV per molecule and the other exponent, which dominates at temperatures below 290 K, was found to be $0.9 \pm 0.5 \text{ eV}$ per molecule.

Table 1 Sources of data used to compute theoretical absorption spectra of monomeric water vapour in the range 0–50 cm⁻¹

Line shape	Gross ⁹ , using expression given by Zhevakin and Naumov ¹⁰ and corrected by Emery ¹¹
Centre frequencies	McClatchey <i>et al.</i> ¹²
Line intensities	
Air-broadened line widths	Benedict and Kaplan ¹³
Self-broadened line width	
Ratio of air-broadened to nitrogen-broadened line widths	
Criteria for inclusion in computation:	
(1) 117 lines in the interval 0 to 50 cm ⁻¹	Lines which, at their peak, contribute 0.19 dB km ⁻¹ or more absorption at 26.5 torr when broadened by 1 atmosphere of air at 300 K
(2) 157 lines in the interval 50 to 700 cm ⁻¹	Lines which contribute 4 × 10 ⁻³ dB km ⁻¹ or more absorption at 50 cm ⁻¹ in above conditions
	(There are no lines in this category above 303.11 cm ⁻¹)

condensed water on the cavity walls giving a change of reflectivity. This was investigated by repeating some measurements in a cavity with a lower surface to volume ratio but these showed no inconsistencies attributable to surface effects. Another error might have come from the small residual standing wave pattern which existed despite the use of the scrambler and which changed with gas pressure. Allowance for this was made in a correction derived from independent measurements on inert gas mixtures and chosen to match the refractive indices and pressures of the gas samples. This correction, which was very small, was only necessary over a limited region in the middle temperature range.

These results show that the absorption of radiation in the 7 cm⁻¹ region by water vapour cannot be fully explained in terms of known properties of monomers and equilibrium dimers. At low temperatures an additional process which as yet can only be loosely described as non-equilibrium seems to be important and to affect both laboratory and field measurements. This has the important consequence that it should now be possible to do systematic, controlled experiments on anomalous absorption previously investigated mainly in the atmosphere. For these experiments the type of nonresonant cavity described here has shown itself to be a marked improvement on conventional absorption cells¹⁸.

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On the occurrence of phosphosilicates

TOFIELD *et al.*¹, in describing the preparation of VO(P₂SiO₈) suggested that 'condensed phosphosilicates may be a more extensive class of materials than has previously been suspected'. Hitherto only two compounds have been shown by structure determination to contain condensed phosphosilicate anions: VO(P₂SiO₈) itself^{2,3}, which contains linked silicate and phosphate tetrahedra, and (NH₄)₂SiP₄O₁₃ (ref. 4), in which the silicon is in octahedral coordination. The synthesis of feldspars containing phosphorus has recently been reported⁵, some of which must contain Si–O–P links. The only other established occurrences of such links are in the silicon phosphates^{6–8}. We describe here our attempts to prepare further examples of phosphosilicates.

An examination of the data in ref. 9 showed that the only system in which potential condensed phosphosilicates had been reported was Na₂O–P₂O₅–SiO₂ (ref. 10). These were 9Na₂O · 2P₂O₅ · 6SiO₂ (9:2:6) melting congruently at 1,100 °C, and 5Na₂O · P₂O₅ · 4SiO₂ (5:1:4) decomposing incongruently at 953 °C. 15Na₂O · 5P₂O₅ · 6SiO₂ (15:5:6) was also observed as a peritectic compound produced by reacting (9:2:6) with 2Na₂O · P₂O₅. More recently the existence of further compounds in this system along the join Na₃PO₄–Na₂SiO₃ has been claimed¹¹, although earlier workers¹² found no evidence of these, or of any other ternary compounds.

Accordingly, we prepared mixtures corresponding to the first two compounds reported in ref. 10, and two intermediate compositions ([1] 84.3 mol % Na₂SiO₃:15.7 mol % Na₃PO₄ and [2] 25.1 mol % Na₂SiO₃:74.9 mol % Na₃PO₄) on the join reported in ref. 11. In both cases a variety of techniques and starting materials was used.

In attempts to prepare the compounds 9:2:6 and 5:1:4 (ref. 10) the starting materials used were Na₃PO₄+Na₂Si₂O₅; Na₄P₂O₇+Na₂SiO₃+SiO₂; and glasses prepared from both these mixtures. These were heat-treated at temperatures between 825 °C and 1,300 °C for periods varying from 1 h to 10 d. Samples were examined by optical microscopy and by X-ray diffraction using a Philips PW 1050/25 diffractometer. There was no evidence of phosphosilicate formation. The products were either a mixture of Na₃PO₄ and Na₂Si₂O₅, or glass, according to conditions of treatment.

Attempts to prepare compounds on the join Na₃PO₄–Na₂SiO₃ were equally unsuccessful. The initial crystalline materials were recovered unchanged from mixtures heated below the reported melting points (1,120 °C for [1] and 1,020 °C for [2]); glasses prepared from them decomposed on prolonged annealing at 825 °C to give again a mixture of Na₃PO₄ and Na₂SiO₃.

Attempts to prepare ternary compounds by precipitation from aqueous solutions were also unsuccessful. We were therefore unable to confirm the reports of the existence of sodium phosphosilicates and conclude that, if such compounds exist, they can be prepared only with extreme difficulty and must be metastable, at least at temperatures below 825 °C.

It seemed possible that phosphosilicates of more electro-negative elements might be more readily prepared; no phase equilibrium data for such systems are available⁹. However, trial runs using mixtures of CdSiO₃ with Cd(PO₃)₂ and PbSiO₃ with Pb(PO₃)₂ produced no evidence of phosphosilicate formation. The products in both cases were mixtures of the orthophosphate and low cristobalite.

From the above results, we conclude that condensed phosphosilicates cannot be prepared from compositions at the

metasilicate–metaphosphate ratio, nor from more basic mixtures. Reports of the occurrence of compounds in this region of the system $\text{Na}_2\text{O}-\text{SiO}_2-\text{P}_2\text{O}_5$ are erroneous. In view of the formulae of the anions known to exist ($\text{P}_2\text{SiO}_8^{2-}$ and $\text{SiP}_4\text{O}_{13}^{2-}$) it seems that any further examples of phosphosilicates must be sought at relatively acidic compositions.

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Mean residence times in steady-flow and some non-flow systems

WHEN an incompressible fluid flows at a constant rate through a constant-volume system, the mean residence time of the fluid elements is equal to the ratio of the volume to the volumetric flow-rate and does not depend on the nature of the flow pattern. This possibly surprising result is of value in several branches of science and technology: it was brought to the attention of chemical engineers by Danckwerts, and is associated in the biological literature with Stewart and Hamilton^{2,3}. Early proofs assume that the fluid, having entered the system, may leave only through the exit and, having left, cannot return. Such a system is said by chemical engineers to be closed. Gibilaro has recently found^{4,5} that the mean residence time in a region of a flow system is the ratio of the volume of that region to the volumetric flow-rate through the entire system. The new result differs from the previous one in that fluid may pass through the region several times, or not at all, before leaving the system, and in that the region may be connected to the system in any way. Residence time is counted as the total time a molecule spends in the region before ultimately leaving the system, and the average is taken over all molecules entering the system, including those which never enter the region. Gibilaro also emphasised the diversity of possible applications: the mean residence time is of interest in the study of continuous chemical reactors, respiratory systems, rivers and so on. Washout experiments are used in biological studies⁶ but rarely in engineering. In a washout test, tracer is added continuously to the feed stream until steady conditions prevail. Tracer flow is then stopped; the inventory of tracer in the system recorded as a function of time is the washout function. This is related to the residence-time density and distribution functions of closed systems⁷ and may also be used in studies of non-closed (open) systems⁸. Here we show how the washout-function idea may be used as the basis of a mean-residence-time theorem of the same type as Gibilaro's but of wider compass: it can apply to non-flow, as well as continuous-flow systems.

Consider a system in the steady state with respect to the transmission through it of one or more species. Transmission may be by flow or by some other mechanism such as diffusion through an otherwise stagnant medium. The i th species is transmitted through the system at mass rate R_i from some source. At time zero, the flow of i from the source is replaced

by i' which is distinguishable from i but otherwise identical. The mass of i in a designated region of the system at time t is the washout function, $g_i(t)$, for that region.

Now consider the case where the flow of i from the source is a pulse:

$$\begin{aligned}\text{Flow of } i &= 0, & t \leq 0 \\ &= R_i, & 0 < t \leq \Delta t \\ &= 0, & \Delta t < t\end{aligned}$$

Here R_i has the same value as it had for the washout experiment and i' flows at rate R_i when the flow of i is zero. The pulse can be thought of as a flow at rate R_i lasting over the interval $(-\infty, \Delta t)$ from which is deducted a flow of R_i lasting over $(-\infty, 0)$. As i and i' behave in the same way, the mass of i present in the region at time t as a result of the pulse injection is that which would be present if steady flow stopped at time Δt less that which would be present if steady flow stopped at time zero. The holdup of i in the region, per unit mass injected at the source, is $[g_i(t - \Delta t) - g_i(t)]/R_i \Delta t$.

In the limit as $\Delta t \rightarrow 0$ we obtain the impulse response, the response of the region to instantaneous injection of one mass unit of i against a background of i' .

$$\phi_i(t) = -\frac{1}{R_i} \frac{dg_i}{dt} \quad (1)$$

In the steady state, the mean period spent in the region by the molecules that comprise any particular batch of i leaving the source will be the same as that spent by any other. To find the mean residence time of i in the region it suffices to find the mean residence time for one batch. When unit mass of i leaves at $t = 0$, the total 'molecule-hours' of contact between i and the region is

$$\frac{N_A}{M_i} \int_0^\infty \phi_i(t) dt \quad (2)$$

where N_A is the Avogadro number and M_i is the molecular weight of i . The mean residence time follows by dividing expression (2) by the number of i molecules leaving the source, N_A/M_i . In view of the relation between ϕ and g , equation (1), and as the region will eventually be clear of i molecules in a washout test ($g_i(\infty) = 0$), the mean residence time is

$$\bar{t}_i = g_i(0)/R_i \quad (3)$$

This is the ratio of the steady-state holdup of i in the region in question to the steady rate of transmission of i through the system, and is of exactly the same form as a rule deduced previously⁸ for closed systems. Molecules are given as something to count: equation (3) applies to any conserved entity. Some examples of the use of this result follow.

If a single-phase system has only one inlet and one outlet through which a homogeneous fluid passes in bulk flow only, the transmission rate is the product of the flow-rate, Q , through the system and the fluid density, while the region holdup is the product of its volume, V , and the density. The region mean residence time follows from equation (3) and is

$$\bar{t} = V/Q \quad (4)$$

Equation (4) applies to a section of finite length of an infinitely long pipe carrying a fluid in which axial mixing takes place: the source may be imagined to be infinitely far upstream. A more interesting example is where another substance, i , is injected into the stream, say at $x = 0$. Suppose that the flow of i is small and that the flow in the pipe is axially dispersed plug flow. Then the steady-state concentration, c , of i obeys:

$$D \frac{d^2 c}{dx^2} - U \frac{dc}{dx} = 0 \quad (5)$$

D is a dispersion constant and U the stream velocity, which is constant because the added flow is small. A long way upstream

c is small and downstream c remains finite. As mixing takes place in the flow, c is continuous at $x=0$. The appropriate solution of equation (5) is

$$\begin{aligned} c &= c_0 \exp(Ux/D) & \text{when } x < 0 \\ c &= c_0 & \text{when } x \geq 0 \end{aligned} \quad (6)$$

where c_0 depends on the rate of addition of i . For pipe sections downstream from $x=0$ the mean region residence time of i is V/Q , by the same argument as before. The mean residence time for sections of pipe upstream from the injection point may also be calculated. Indeed the mean residence time in the semi-infinite upstream section is finite: by integration of equation (6) we find that $g_i(0)=c_0 D/U$, while the system transmission rate is Uc_0 . Hence

$$\bar{t}_i = D/U^2 \quad (7)$$

As an example of a mean residence time in a stationary medium, consider steady one-dimensional diffusion between a source at $x=L$ and a sink at $x=0$. The concentration profile is $c=c(L) \cdot x/L$ which gives a steady state holdup (per unit cross-sectional area) of $\frac{1}{2}Lc(L)$. The transmission rate (again per unit c.s.a.) is $\mathcal{D}c(L)/L$, $\mathcal{D}$ being the diffusivity. Applying equation (3), the mean residence time between $x=L$ and $x=0$ is

$$\bar{t}_i = L^2/2\mathcal{D} \quad (8)$$

The mean residence time for any intermediate section may be found in the same way.

We conclude with an example in which the mean residence time is finite in part of an infinite stationary medium. Substance i is generated in a spherical void, radius r_0 , and steadily diffuses away in all directions. The diffusion equation

$$\frac{d}{dr} \left(r^2 \frac{dc}{dr} \right) = 0 \quad (9)$$

has a solution $c=c_0 r_0/r$ which tends to zero at large distances from the void. Here c_0 is the concentration at r_0 . The transmission rate is equal to the rate of diffusion from the void and is $4\pi\mathcal{D}r_0 c_0$. The holdup in the zone $r_0 < r < r_1$, obtained by integration, is $2\pi c_0 r_0 (r_1^2 - r_0^2)$, and thus the mean residence time in this zone is

$$\bar{t}_i = (r_1^2 - r_0^2)/2\mathcal{D} \quad (10)$$

Notice that equation (10) tends to

$$\bar{t}_i = r_1^2/2\mathcal{D} \quad (11)$$

as r_0 tends to zero; now $\bar{t}$ can be thought of as the mean residence time in the neighbourhood of a point source from which material is dispersed by diffusion.

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α' -Sialon ceramics

OUR previous report¹ on Si-Al-O-N ceramics stated that expanded α -silicon 'nitride' structures had been obtained by reaction of lithium-silicon nitride, LiSi_2N_3 , with alumina. The unit-cell dimensions of one example (a , 7.822; c , 5.677 Å) gave

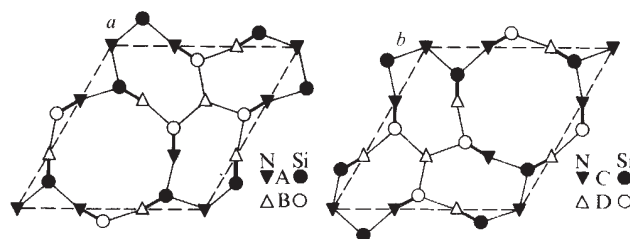


Fig. 1 Idealised Si-N layers in α and β silicon nitrides: a, AB layers; b, CD layers. β -structure, ABAB; α structure, ABCD.

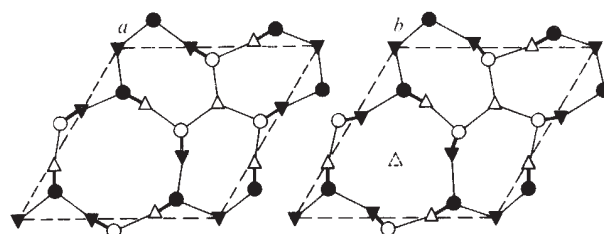


Fig. 2 Actual Si-N layers in α and β silicon nitrides: a, in β ; b, in α .

a cell volume about 3% greater than that of α -silicon nitride. Subsequent work² showed a variation in dimensions for the α' -lithium sialon when lithium aluminate, LiAlO_2 , was reacted in different proportions with Si_3N_4 . However, other phases, such as β' -sialon and nitrogen-eucryptite, were always present and the product never contained more than ~30% of the α' material. $\alpha\alpha'$ -Sialons were also found in the Mg-Si-Al-O-N system³ but again never pure, and were observed by Masaki *et al.*⁴ during the nitriding of silicon with AlN and Al_2O_3 additions. A recent claim by Mitomo⁵ of α' solid solutions of Si_3N_4 - Al_2O_3 and/or Si_3N_4 - Y_2O_3 occurring during the sintering of Si_3N_4 at 1,700-1,800 °C with Al_2O_3 - Y_2O_3 mixtures prompted this report of the preparation and characterisation of pure α' -phases in M-Si-Al-O-N systems where M is Li, Ca or Y. Such phases also occur, but have not so far been prepared in a pure form, in magnesium and other sialon systems.

The 'idealised' silicon nitride structure can be described as a stacking of Si-N layers in either an ABAB... (β) or an ABCD... (α) sequence as shown in Fig. 1. This gives long continuous channels in β , parallel to the hexagonal c -direction and centred at 2/3, 1/3 in the outlined cell of Fig. 1a. In α , the c -glide plane relating the layers CD with AB replaces the continuous channels of the β structure by large closed interstices at 1/3, 2/3, 3/8 and 2/3, 1/3, 7/8. In the α unit cell containing, ideally, Si_2N_{16} there are therefore two sites large enough to accommodate other atoms or ions. Although the Si-N layers in the actual β structure are almost identical with the 'ideal' configurations (see Fig. 2), those of α are considerably distorted and nitrogen atoms at heights approximately 3/8 and 7/8 are shifted towards the centres of the two respective interstices.

All the α' -sialons have compositions represented by $\text{M}_x(\text{Si}, \text{Al})_{12}(\text{O}, \text{N})_{16}$ where $x \geq 2$. Cell dimensions of examples where $x \geq 1$, are compared with those of α and β silicon nitrides in Table 1. There are small but distinct differences between the X-ray diffracted intensities of α and α' and a complete structure determination for a composition near $\text{Ca}[\text{Si}_9\text{Al}_3\text{ON}_{15}]$ shows that each of the two interstitial sites contains on average half a Ca atom.

The α' -structure is derived from α - $\text{Si}_{12}\text{N}_{16}$ by partial replacement of Si with Al. Valency compensation is effected by the 'modifying' cations (Li, Ca, Y) occupying the interstices of the (Si, Al)-N 'network' but where a modifier oxide is used, oxygen may also replace nitrogen. When α' is synthesised entirely from nitrides, for example, a mixture of Si_3N_4 , AlN and Ca_3N_2 , the product contains no oxygen, and valency

Table 1 Unit-cell dimensions and densities of α' -sialons and α and β silicon nitrides

	<i>a</i>	<i>c</i>	<i>c/a</i>	<i>d_o</i>	<i>d_c</i>
β -Si ₃ N ₄	7.61	2.91	0.765/2	3.192	3.192
α -Si ₃ N ₄	7.76	5.62	0.724	3.16*	3.183
LiSi ₁₀ Al ₂ ON ₁₅	7.83	5.67	0.724	3.12	3.14
Ca _{0.5} Si _{10.5} Al _{1.5} O _{0.5} N _{15.5}	7.82	5.68	0.727	3.16	3.20
Ca _{0.8} Si _{9.2} Al _{2.8} O _{1.2} N _{14.8}	7.86	5.71	0.727	3.19	3.26
Y _{0.4} Si ₁₀ Al ₂ O _{0.8} N _{15.2}	7.81	5.69	0.729	3.23	3.25
Y _{0.6} Si _{9.2} Al _{2.8} O _{1.1} N _{14.9}	7.83	5.71	0.729	3.28	3.36

d_o, observed density; *d_c*, calculated density;

* Range of values 3.167–3.171 g ml⁻¹ obtained by Wild *et al.* for α -needles produced by SiO–N₂ reaction⁶.

compensation is due solely to the introduction of modifying cations. Because there are only two sites per unit cell for these, the limiting composition for Ca- α' -nitride is Ca₂Si₈Al₄N₁₆. The limit for the corresponding oxynitride is also two calciums per unit cell but the extent of the replacement of silicon by aluminium and oxygen by nitrogen has not yet been determined.

The α' -sialons are prepared by heating appropriate mixtures of nitrides or nitrides plus oxides at 1,750 °C in one atmosphere of molecular nitrogen or argon for 15 min. Weight losses are negligible and the composition of the initial mix, calculated from the proportions of powder constituents with allowance for surface oxide on the nitrides, is virtually identical with direct microanalysis of the product using a Camebax electron probe at AERE, Harwell. Moreover, densities calculated from compositions, cell dimensions and the proposed structures are, as shown in Table 1, in excellent agreement with the observed densities.

Typical X-ray photographs of Ca, Li and Y α' -sialons are shown in Fig. 3 from which it is clear that exactly the same product is obtained starting with β -Si₃N₄ as with α -Si₃N₄. Unlike the β' -sialons, Si₆₋₂Al₂O₂N₈₋₂, where the replacement without structural change is (Si–N) by (Al–O), the replacement in α' -phases is largely (Si–N) by (Al–N). With bond lengths Si–N ~ 1.74, Al–O ~ 1.75 and Al–N ~ 1.87 Å, the relative increases in unit-cell dimensions for $\alpha \rightarrow \alpha'$ are much greater than for $\beta \rightarrow \beta'$. For a general composition

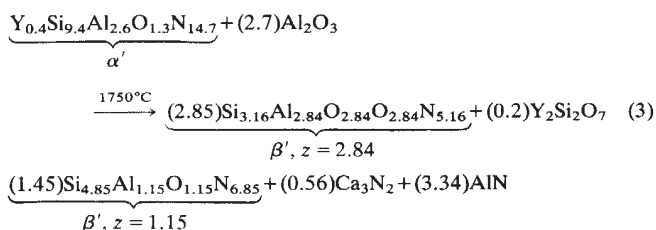


there are *m*(Si–N) replaced by *m*(Al–N) replaced by *n*(Al–O). If the unit-cell dimensional changes corresponding to these respective replacements are in the ratio 5:1, Fig. 4 shows that the observed dimensions for a range of α' -phases fit the relationships:

$$\Delta a (\text{\AA}) = 0.045m + 0.009n \quad (1)$$

$$\Delta c (\text{\AA}) = 0.04m + 0.008n \quad (2)$$

The X-ray photographs of Fig. 5 show that α' reacts with Al₂O₃ to give β' and β' reacts with AlN plus the appropriate modifier nitride (such as Ca₃N₂) to give α' . Equations corresponding to these reactions are:



Just as β' -sialons can be produced by nitriding mixtures of oxides and carbon with molecular nitrogen, so also can these

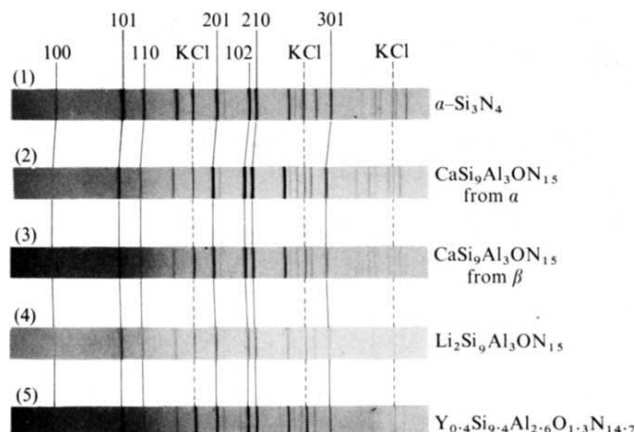


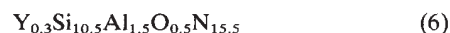
Fig. 3 Typical X-ray powder patterns of Ca, Li and Y α' -sialons. CuK α radiation; Hagg–Guinier focusing camera.

α' -phases. Calcium compounds have been prepared in this way at temperatures as low as 1,400 °C. Technological advantages of α' compared with β' sialons are not yet known but it seems that the field for exploration has been considerably widened.

The α' compositions so far observed that are closest to Si₃N₄ are



and



If it is accepted that α' requires the equivalent of at least half a cationic valency (Ca_{0.25} or Y_{0.16}) in each of the two interstices to stabilise the structure, it is suggested that this is also the requirement in 'pure' α -silicon nitride. The corresponding compositions are then



and



depending on whether oxygen is available for valency compensation. It is assumed that only Si³⁺, and not Si⁴⁺, is large enough to be accommodated in the structural interstices; the observed lengths of 2.2 Å for these Si–N bonds seem reasonable. Certainly, there seems no reason for the structural distortion of α and α' other than partial occupation of such sites. It is hardly fortuitous that the calculated density for the α composition, equation (8), 3.170 g ml⁻¹, almost exactly agrees with the density range observed⁶ for α needles produced by reaction of silicon monoxide with nitrogen, 3.167–3.171 g ml⁻¹, and which cannot be explained by the composition Si₃N₄. The unit-cell dimensions determined by one of us (D.P.T.) for 26 different α -silicon nitrides prepared by (1) reacting Si with N₂; (2) the reaction of SiO with N₂; and (3) chemical vapour deposition from silicon halides, vary widely within limits *a* = 7.7491–7.7619 and *c* = 5.6151–5.6221 Å.

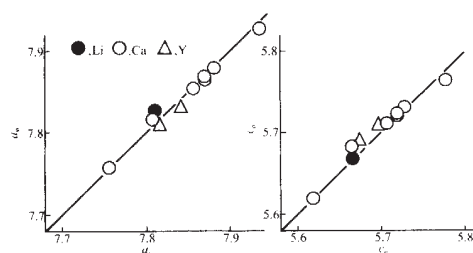


Fig. 4 Observed (o) and calculated (c) unit-cell dimensions for α' -sialons $M_xSi_{12-(m+n)}Al_{(m+n)}O_nN_{16-n}$; *m* = 1–4; *n* = 0–2.5. $\Delta a_c = 0.045m + 0.009n$ Å; $\Delta c_c = 0.04m + 0.008n$ Å. Si–N, 1.74 Å; Al–N, 1.87 Å; Al–O, 1.75 Å.

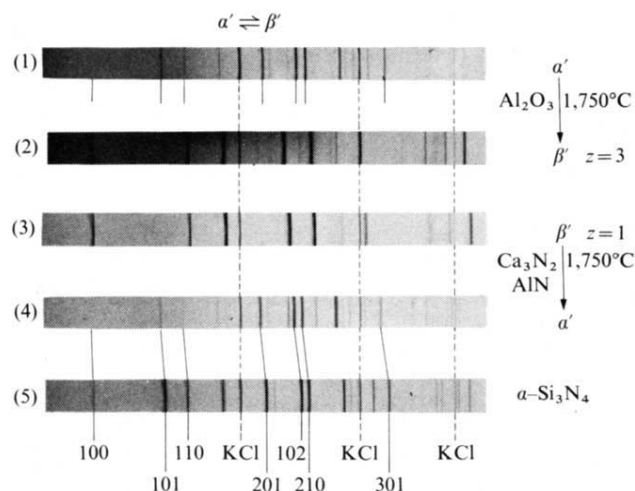


Fig. 5 X-ray powder patterns showing transformations $\alpha' \rightleftharpoons \beta'$ CuK α radiation; Hägg-Guinier focusing camera.

Considering that the same precise method was used in each case by the same investigator, this variation is well outside any experimental error ($\pm 0.0005 \text{ \AA}$) and is thought to be due to variation within the range of compositions given by equations (7) and (8). It is further suggested that the transformations $\alpha \rightleftharpoons \beta$ and $\alpha' \rightleftharpoons \beta'$ take place only by liquid-phase or vapour-phase processes. Further details will be published elsewhere.

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The Rankine-Hugoniot relation for shock waves in very porous media

AN entropy rise accompanies shock wave propagation in any medium. The entropy jump at a shock front is especially high when a medium contains a large number of pores—small empty holes randomly distributed. Shock compression of a porous medium leads to intense heating of the material surrounding the pores because of its nonelastic deformation. Intense heating of a porous material at a shock front yields an 'anomalous' shape for the Rankine-Hugoniot curve on the p - V plane^{1,2}. This effect of porosity was clearly seen in our experiments with cast foamed polystyrene of various initial densities, ρ_0 . The solid lines in Fig. 1 represent Rankine-Hugoniot curves obtained experimentally. It can be seen that the specific volume of compressed material is much greater than the volume of polystyrene in the condensed (solid or liquid) state. It is clear that such a great increase in volume is possible only when initially condensed porous material turns into the gaseous state, and cannot be explained in terms of thermal expansion.

Photographs by high speed camera (Fig. 2) confirm that shock-wave compression of very porous polystyrene is associated with a transition into the gaseous phase. Figure 2 shows that propagation of a shock wave in porous polystyrene (the arrow in Fig. 1 indicates the corresponding p - V values) is followed by a very intense expansion flow of compressed material. The expansion flow must be related to the gasification of polystyrene, because it was found experimentally that a decrease of air pressure in the pores to $5 \times 10^{-5} \text{ atm}$ had no effect on rarefaction.

The overall pattern of a shock front followed by intense expansion flow of gasified material shows more similarities to a detonation wave than to a shock wave in a homogeneous medium. Experimental data must therefore be explained by analogy with a detonation wave. Thus to correct the Rankine-Hugoniot relation for the case considered we must take into account a phase state transition taking place at a shock front which is (in contrast to detonation) endothermic.

If w , p and V are enthalpy, pressure and specific volume respectively and subscripts o and s denote the states of the material in front of and behind a shock wave, the Rankine-Hugoniot relation may be written in the form

$$w_s - w_o + \lambda_v = \frac{1}{2}(p_s - p_o)(V_s + V_o)$$

where λ_v is the heat of gasification (vaporisation, sublimation, chemical decomposition and so on). In general, p_o and w_o are small in comparison with the other terms of the equation and one may put $p_o = 0$, $w_o = 0$. Enthalpy w_s can be expressed by one of the equations of state for dense real gases. For a gas at moderate density,

$$pV = RT \left(1 + \frac{b}{V} \right)$$

which gives for w_s

$$w_s = c_p T = \left(\gamma \frac{p_s V_s}{\gamma - 1} \right) / \left(1 + \frac{b}{V} \right)$$

(R is the gas constant; T , temperature; b , second Van-der-Waals constant; $\gamma = c_p/c_v$; 'Poisson's ratio'). Substituting w_s in

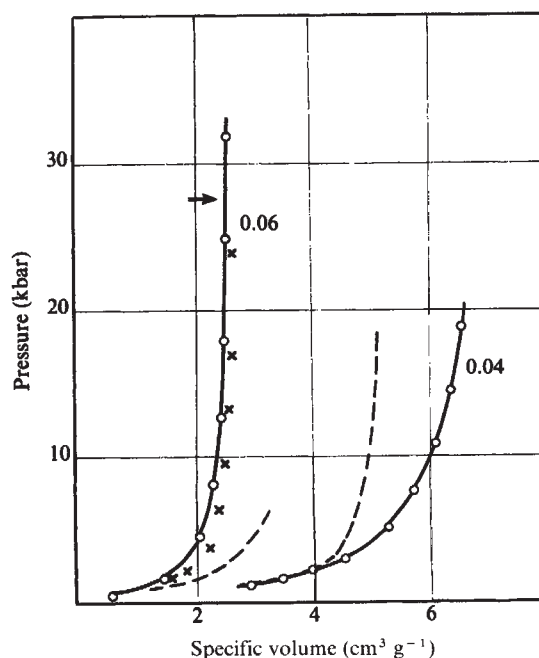


Fig. 1 Experimental (solid lines) and computed (broken lines and crosses) Rankine-Hugoniot curves for cast foamed polystyrene of low density. Values shown against lines are initial densities in g cm^{-3} (ref. 2).

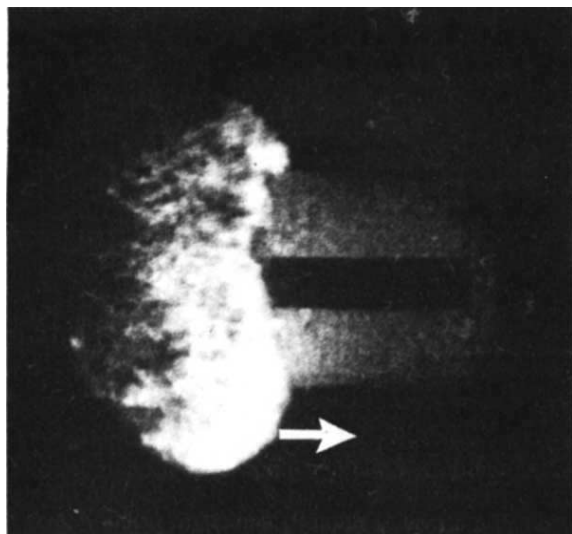


Fig. 2 Rapid expansion of porous polystyrene compressed in shock wave. The arrow denotes the direction of shock propagation, dark band is a part of scaling type located on the incompressible material. Note the intense self-luminosity of the expanding material. (Magnification $\times 0.58$).

the above Rankine-Hugoniot equation yields the expression for p_s :

$$p_s = -\lambda_v \left[\frac{\gamma V_s^2}{(\gamma-1)(V_s+b)} - \frac{1}{2}(V_o + V_s) \right]^{-1}$$

From this equation we obtain for the Rankine-Hugoniot curve a vertical asymptote $V = V^*$ and

$$V^* = \frac{(\gamma-1)(V_o+b)}{2(\gamma+1)} + \left\{ \left[\frac{(\gamma-1)(V_o+b)}{2(\gamma+1)} \right]^2 + \frac{\gamma-1}{\gamma+1} b V_o \right\}^{1/2}$$

Only that part of the V axis where $V < V^*$ has physical meaning because both numerator and denominator in an expression for p_s must be negative to fit the obvious condition $p_s > 0$. Thus 'anomalous' shapes of Rankine-Hugoniot curves are a result of endothermic processes inherent to shock compression of the porous media.

The derived relation for p_s is in a good agreement with the experimental results. To apply the equation for p_s to shock waves in foamed polystyrene let us estimate λ_v as heat of full thermal decomposition of styrene according to equation: $C_6H_5CHCH_2 \rightarrow 8C + 4H_2$ that gives $\lambda_v = 7.43 \times 10^{10}$ erg g $^{-1}$. Covolume b is equal to 1.02 cm 3 g $^{-1}$ for hydrogen. The results of calculation of p_s with the constants chosen and $\gamma = 1.4$ for $\rho_o = 0.04$ and 0.06 g cm $^{-3}$ are shown as broken lines in Fig. 1.

Poisson's ratio $c_p/c_v = \gamma$ is equal to 1.4 for a gas with two-atom molecules, such as H_2 . If the products of thermal decomposition contain significant amounts of more complicated molecules (for example CH_4) the value for γ becomes less than 1.4 and can be taken as approximately equal to 1.25. Crosses in Fig. 1 represent data calculated for $\rho_o = 0.06$ g cm $^{-3}$ with unchanged λ_v and b , but with $\gamma = 1.25$.

Comparison of the calculated and observed data shows that the 'detonation' approach to shock waves in very porous media provides useful information even if very rough numerical approximations are made. To obtain more accurate analytical results it will be necessary not only to use a proper equation of state for dense gases but also to take into account the thermodynamic equilibrium condition that strengthens the analogy between this type of shock wave and detonation waves.

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Isotopic composition of sulphur in atmospheric precipitation around Sudbury, Ontario

THE isotopic composition of ombrogenic sulphur has been used, with varying degrees of success, to fingerprint sources of sulphur in the atmosphere¹⁻⁴. The major unresolved problem in applying this potentially powerful technique to environmental studies has been the fractionation of sulphur isotopes by the various SO_x washout phenomena. We report here the concentrations and isotopic compositions of sulphur in bulk precipitation samples collected during 1975 and 1976 at stations around the nickel smelting complex at Copper Hill near Sudbury, Ontario. By combining the data with isotopic measurements on sulphur oxides (SO_x) in the smelter stack and plumes, the isotopic effects and the principal mechanisms of sulphur deposition close to the smelter have been evaluated.

The deteriorative effects of smelter emissions on ecosystems around Sudbury have been widely reported⁵⁻⁸. The 381-m smelter stack at Copper Hill is one of the tallest and largest SO_2 point sources in the world, emitting about 3.5×10^6 kg of SO_2 daily⁹, or roughly 20% of all the SO_2 emitted annually in Canada¹⁰. Although impingement on the ground occurs fairly frequently during spring and summer, the plumes generally travel coherently for several kilometres downwind⁹. All the monitoring stations for this study are located at distances less than 100 km from the stack and hence come under the direct influence of plumes from the smelter.

The monthly bulk precipitation samples were obtained using samplers as described by Shiomi and Kuntz¹¹. The methods used in determining the sulphate content of samples, in converting sulphate to SO_2 and in mass spectrometric analysis of ^{34}S : ^{32}S ratios in samples have been described elsewhere¹²⁻¹⁴. The isotopic results are given in the usual $\delta^{34}S$ notation:

$$\delta^{34}S(\text{‰}) = (R_{\text{sample}}/R_{\text{std}} - 1)1,000$$

where R_{sample} and R_{std} refer to the ^{34}S : ^{32}S ratios of the sample and the standard Canyon Diablo troilite respectively. Based on the replicate analyses of several rainwater samples, the overall precision of the $\delta^{34}S$ data reported has been established as $\pm 0.2\text{‰}$.

The striking feature of the ombrogenic $\delta^{34}S$ data (Table 1) is the pronounced seasonal variation, with the rain samples in winter being heavier than those of the summer. For any given month during 1975 and 1976, the isotopic composition of precipitation samples within 100 km radius of the stack is fairly uniform (Table 1). From the mean data for all the stations, the difference in $\delta^{34}S$ values between the winter and summer months is 2-3‰. The sulphate contents of the samples also show a marked seasonality (Table 2) with higher levels in the summer months. The inverse relation between the $\delta^{34}S$ and sulphate concentration suggests that the temporal trend in $\delta^{34}S$ data is related to seasonal differences in the amount and nature of sulphur removed from the atmosphere (see below).

The $\delta^{34}S$ of ombrogenic sulphur is also related to the isotopic composition of ambient SO_x . Samples of SO_2 in the smelter stack and plumes were obtained between 10 and 19 June 1975 and subsequently analysed isotopically (Table 3). Included in Table 3 are the isotopic data reported by Forrest and Newman¹⁵ for plume samples obtained in September 1974. The remarkable feature in Table 3 is the constancy of $\delta^{34}SO_2$ in the stack and plumes. The similarity in $\delta^{34}S$ values during 1974 and 1975 may not be a coincidence; variations in the isotopic

Table 1 Isotopic composition of sulphur in precipitation around the smelting complex at Copper Hill near Sudbury, Ontario

Location	Distance from Sudbury (km)	Jan 1975	Jul–Sept 1975	Sept–Oct 1975	Oct–Nov 1975	Nov 1975	Dec 1975	Jan 1976	Jul–Aug 1976	Aug–Sept 1976	Sept–Oct 1976
Sudbury South		+5.80	+4.25	+4.82		+3.18	+5.80	+6.79	+2.89	+3.47	+5.71
Skead	27, NE	+5.71	← +4.74 →		+4.84			+6.33	+4.32	+4.48	+4.63
Windy Lake	35, WNW	+5.35	+4.47	+3.96	+4.87	+5.75	+6.06	+6.72		+3.80	3.16
Jamot	53, SE	+5.50	+4.30	+3.96			+5.22	+6.11	+2.04	+3.67	+4.64
Verner	63, E	+6.10	+5.14		+3.76	+5.06	+5.92	5.64			
Killarney	75, SW	+5.35	+5.08	+3.63			+6.08	+5.54	+2.71	+3.89	+5.00
Laundry L.	48, NE									+4.40	+5.00
Welcome	90, N									+4.92	+4.18
Average (all stations)		+5.64	+4.66	+4.22	+4.49	+4.66	+5.82	+6.19	+2.99	+4.09	+4.62

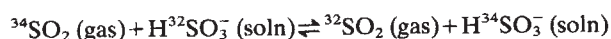
Table 2 Sulphate concentrations (p.p.m.) in precipitation samples around Sudbury, Ontario

Sulphate concentration and sampling time										
Location	Jan 1975	Jul–Sept 1975	Sept–Oct 1975	Oct–Nov 1975	Nov 1975	Dec 1975	Jan 1976	Jul–Aug 1976	Aug–Sept 1976	Sept–Oct 1976
Sudbury South	2.3	8.4	7.8	11	7.2	4.4	2.4		3.7	4.3
Skead	2.1	← 7.4 →		4.1			4.7	9.1	6.2	4.2
Windy Lake	1.2	4.4	7.8	5.5	3.2	3.2	1.7	4.4	2.2	5.0
Jamot	2.2	4.7	6.0		2.2	2.2	1.7	4.6	4.9	3.4
Verner	1.6	4.4	8.1	6.2	3.6	2.8	2.6			
Killarney	1.6	5.5	3.0		2.7	2.8	1.8	4.6	4.9	3.4
Laundry Lake									3.3	3.6
Welcome									3.4	3.1
Average (all stations)	1.8	5.8	6.7	6.7	3.8	2.9	2.5	5.7	4.1	3.8

composition of ore feeds are relatively small¹⁶. Sudbury is thus a suitable area for studying the isotopic effects of the various SO_x precipitation mechanisms.

A comparison of Tables 1 and 3 shows that the precipitation samples are enriched in ³⁴S relative to the plume and ambient SO₂. The isotopic effects associated with the washout of SO_x in the plume range between 1.0 and 5.3‰ with an annual average of 3.5‰. The similarity in δ³⁴S values of airborne sulphate and those of the precipitation samples is quite striking and suggests that the ombrogenic sulphur was mostly derived from sulphate aerosols. Muller and Kramer¹⁷ estimated that 21 × 10⁶ g of SO₄ aerosols was emitted daily by the smelter stacks and that all the SO₄ so emitted was deposited in the vicinity of the smelter. We have analysed three SO₄ samples obtained straight from the tall stack and obtained a mean δ³⁴S value of 3.7‰, which is consistent with the generic affinity suggested for the sulphur in the precipitation samples.

Some of the sulphate deposited in the Sudbury region (about 16 × 10⁶ g per day or 0.6% of the SO₂ emitted, see ref. 17) is derived from the oxidation of SO₂ to sulphate. Several studies^{9,15} have shown that the oxidation rate for SO₂ in Sudbury plumes is quite low with 1% h⁻¹ being the reported representative average⁴. The low rate of SO₂ oxidation would imply that the fractionation of sulphur isotopes due to the rate controlled oxidation reactions (see ref. 18) would be small. In such a situation, it is conceivable that the isotopic effect due to the rate controlled oxidation of SO₂ may just be in balance with exchange reactions such as



which enrich the sulphate aerosols with ³⁴S (see Table 3). Such isotope fractionation scheme would be consistent with the increase in sulphate concentration as well as the reduction in δ³⁴S values observed during the summer, which may be attributed to a higher rate of SO₂ washout by rainfall during the summer.

Table 3 Isotopic composition of stack and plume samples: a, June 1975; b September 1974

	Sample, distance from stack (km)	Plume age (min)	$\delta^{34}\text{SO}_2$ (‰)	$\delta^{34}\text{SO}_4^{2-}$ (‰)
<i>a</i> *				
10	Stack	—	+1.15	—
11, am	3.2	6.5	+1.08	—
	96	196	+1.12	—
11, pm	Stack, 1	—	+1.11	—
	Stack, 2	—	+0.93	—
17	Stack	—	+1.06	—
	42	52	+0.99	—
18	3.2	6.4	+1.32	—
	32	64	+1.14	—
19	Stack	—	+0.98	—
	3.2	8.6	+1.23	—
	24	73	+1.16	—
	56	170	+1.18	—
<i>b</i> †				
Run 1	Background	—	+2.6	—
	1.6	2.2	+1.3	+6.4
	8	11	+1.8	} +6.5
	16	22	+1.6	
	48	67	—	} +7.6
	81	113	+1.4	
Run 2	1.6	2.7	+1.2	+4.2
	8	13	+1.4	+4.2
Run 3	16	—	+1.4	+5.6
	113	—	+1.1	+5.3

* The characteristics of the 1975 plume samples are described in ref. 4.

† Results of 1974 plume experiments are taken from ref. 9.

The super-tall stack installed at Copper Hill in 1972 now broadcasts the stack emissions to a wide area and has substantially reduced the ambient SO₂ levels around Sudbury¹⁹. This

study, however, shows that the preferential deposition of sulphuric acid aerosols occurs close to the smelter and would tend to mitigate the effects of the tall stack on the acidity of atmospheric precipitation around Sudbury. Indeed, Kramer²⁰ has shown that the rate of H^+ deposition in Sudbury area has increased 1.6–3 times since the installation of the tall stack. Tall stacks may not, apparently, always provide the desired reprieve against acid rain problems in the immediate vicinity of the SO_2 point source. In this respect, it is significant that the isotopic composition of the ombrogenic sulphur is very similar to that of the sulphur in poorly buffered lakes around Sudbury²¹.

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A problem in ^{210}Pb geochronologies of sediments

KNOWLEDGE of sedimentation rates in marine and lake deposits is of fundamental importance in understanding aquatic and sedimentary geochemical processes which may have been measurably altered since deposition times. A chronological method based on ^{210}Pb (half life 21.3 yr) is useful in sedimentary environments in which sedimentation rates have been constant

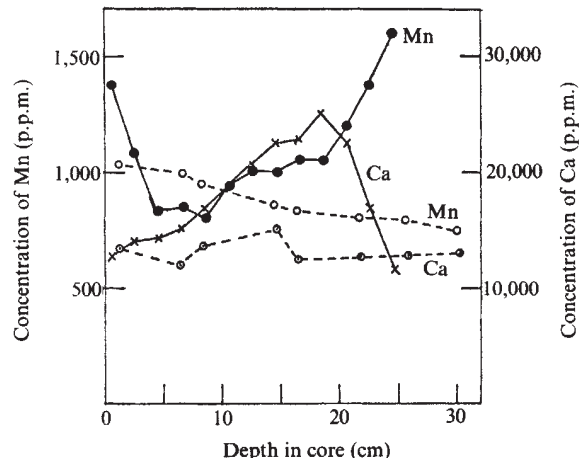


Fig. 2 Concentrations of Ca and Mn in the samples as a function of depth in Lake Suwa (—) and Osaka Bay (---).

and sedimentation strata undisturbed by physical or biological mixing processes^{1–6}. Studies in Lake Suwa and Osaka Bay have revealed, however, an anomaly in the ^{210}Pb concentration in the lower level of the sediments. It is proposed here that the principal controls on ^{210}Pb scavenging from water are both soil particles and hydrous oxides of manganese.

Cores were taken from the deepest part of Lake Suwa with an Ekman–Birge corer and from Uozaki Harbour of Osaka Bay with a giant corer. The former was sliced into 1-cm sections and the latter into 2.5-cm sections. Each dried sample of 11–18 g was packed in a disk-shaped case and subjected to γ -ray spectrometry with a germanium (lithium) detector (16 mm in diameter, 5.13 mm thick). To reduce the background counting rate, the upper part of the detector was surrounded with three shielding materials: 5 cm thick lead, 1 cm thick brass and 0.2 cm thick cadmium⁷. The activity of ^{210}Pb was determined from the area of the photopeak due to its 46.5 keV γ rays^{7,8}. The activity of ^{210}Pb shown in Fig. 1 is unsupported ^{210}Pb ; ^{226}Ra -supported ^{210}Pb having been subtracted from the total ^{210}Pb . More than 30 days after sealing, when radioactive equilibrium between radium and radon was expected to have been established, each sample was again subjected to γ ray spectrometry using a large volume germanium (lithium) detector. The ^{226}Ra contents were determined from the areas of the photopeaks due to the 609 keV γ rays of ^{214}Bi . Apparently the ^{210}Pb content of Lake Suwa in the upper part of the core decreases gradually with depth and increases slightly in the lower part (under 20 cm). ^{210}Pb in the core from Osaka Bay shows a classical exponential decrease with depth, corresponding to a sedimentation rate, derived from a least squares fit, of 0.62 cm yr^{-1} .

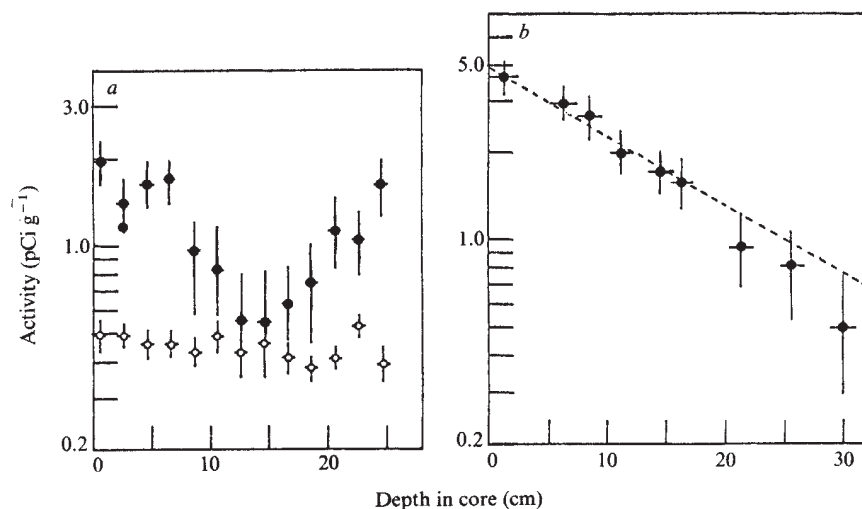


Fig. 1 ^{210}Pb (●) and ^{226}Ra (○) activities as a function of depth: a, Lake Suwa; b, Osaka Bay (rate of deposition 0.62 cm yr^{-1}).

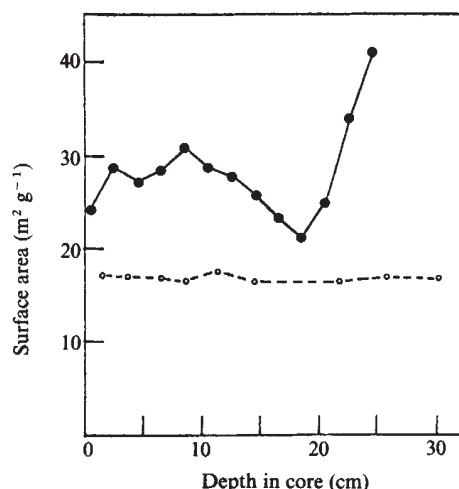


Fig. 3 Surface area of the samples as a function of depth in Lake Suwa (—) and Osaka Bay (---).

The Ca, Mn, Cr, Cu, Fe and K contents in the sample were determined by atomic absorption spectrometry according to the procedure of Bruland *et al.*⁹ and Terashima¹⁰. Figure 2 shows the concentrations of Ca and Mn in the samples as a function of depth. The Ca distribution in the Lake Suwa core shows a maximum at a depth of 18–19 cm. The Mn content is high both at depths over 20 cm and in the top layers of the Lake Suwa sediment, but in the Osaka Bay profile decreases gradually with depth. It is evident that in the Lake Suwa core Cr and Cu concentrations have been significantly influenced by man's activities. There is a much lesser effect in the Osaka Bay core. The Fe and K profiles in both cores show relative uniformity with depth.

Sediment surface area, which is closely related to adsorption and ion exchange properties¹², was determined by rapid surface area analysis using lower-temperature nitrogen adsorption^{13,14}. Figure 3 shows that the surface areas of samples from Lake Suwa vary markedly and increase rapidly with depth in lower sections, while the Osaka Bay profile varies only slightly with depth. In the former core the substantial increases in ²¹⁰Pb and Mn contents at depths over 20 cm are found to be closely related to changes in surface area, and that there is an inverse correlation between the concentration of Ca and surface area.

To identify the cause of the observed variations in surface area, the mineral composition of samples was examined by powder X-ray diffraction¹⁵. X-ray spectra for the samples from Lake Suwa show peaks apparently due to feldspar, quartz and

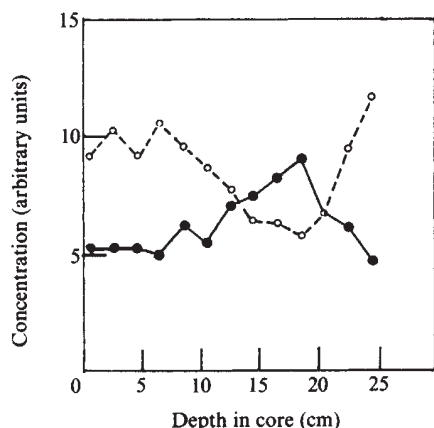


Fig. 4 Contents of quartz (○) and feldspar (●) in the samples collected from Lake Suwa as a function of depth.

Table 1 Linear correlation coefficient for samples from Lake Suwa

	Mn	Surface area	Feldspar	Quartz
Ca	-0.31	-0.48	0.87	-0.94
Mn		0.51	-0.18	0.25
Surface area			-0.60	0.77
Feldspar				-0.87

halloysite, which vary noticeably with depth. For Osaka Bay samples only a slight variation with depth is observed. Figure 4 shows, however, that the quartz content is inversely proportional to that of feldspar (Table 1). In addition, by comparing Figs 3 and 4 the surface area seems to increase with quartz content (Table 1). An explanation of this correlation is that weathering alters feldspars more readily than quartz so that the weathered soil is significantly depleted in feldspars and enriched in quartz¹⁶. Samples enriched in quartz are clearly mainly composed of a fine size soil particles and consequently have a large surface area. The reverse correlation between the concentration of Ca and surface area is therefore explained by the fact that Ca is enriched in feldspars.

Lake Suwa is fed by 26 rivers of which the Kami River, which rises in Mt Yasugadake, is the most important. In 1898 a small landslide occurred on a part of Mt Yatsugadake and the Kami River swelled with muddy water¹⁷. This landslide was therefore

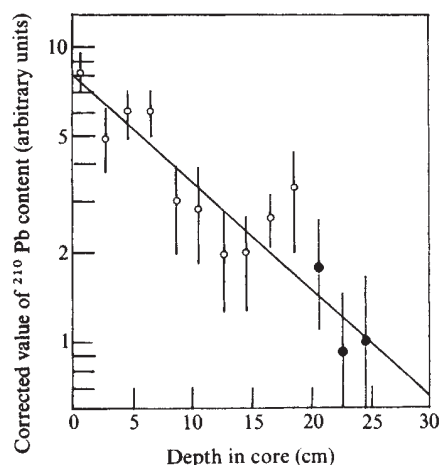


Fig. 5 ²¹⁰Pb activities corrected for surface area as a function of depth (rate of deposition 0.39 cm yr⁻¹).

presumably the cause of the variation in soil particle size of samples from below 18 cm. Thus it is likely that soil particles, deposited by the landslide in the Kami River sediments, were carried into the lake in increasing order of particle size with only small particles reaching the central deeps. After many years the river bottom gradually returned to the original sedimentation pattern, a change also reflected in Lake Suwa sediments.

The sedimentation rate of the sample from Lake Suwa was determined on the upper 20 cm of core by evaluating the vertical decrease in ²¹⁰Pb activity expressed in units of activity per unit surface area. The result of this treatment is a sedimentation rate of 0.39 cm yr⁻¹ as derived in Fig. 5. In treatment of the lower 20 cm, it is surprisingly found that the ²¹⁰Pb contents per unit surface area squared also roughly fit a line corresponding to a sedimentation rate of 0.39 cm yr⁻¹. An explanation of this necessary correction of sub-20 cm data to (surface area)² is that, as evidenced by the high Mn levels at the depth, the majority of the ²¹⁰Pb at depths over 20 cm may have been deposited with hydrous oxides of manganese, which generally precipitate as a coating on clay surfaces¹⁸, as well as with soil particles. Although the Mn concentration is also higher in the upper oxidised zone, this enrichment is generally explained by postdepositional remobilisation^{19,20}.

Scavenging of ^{210}Pb from the water column is classically accepted to be highly efficient and rapid.

It is concluded that the ^{210}Pb contents of sediment samples vary with particle size with Mn content, and with depth. Therefore, when particle size and Mn contents vary with depth, some correction of ^{210}Pb concentration is required for useful chronological studies.

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Photoperiodically determined dimorphic calling songs in a katydid

MALES of most species of katydids (Orthoptera, Tettigoniidae) make calling songs that attract conspecific females^{1–4}. These songs, like those of crickets, cicadas, and some grasshoppers, are species specific and can be produced in perfect form by males that have never heard a similar song⁵. The fact that insect calling songs are the same even though conditions during their development varied from generation to generation and between field and laboratory has led to the inference that the ontogeny of such songs is free of environmental modification⁶. We show here, however, that the calling songs of a katydid are dimorphic and photoperiodically determined.

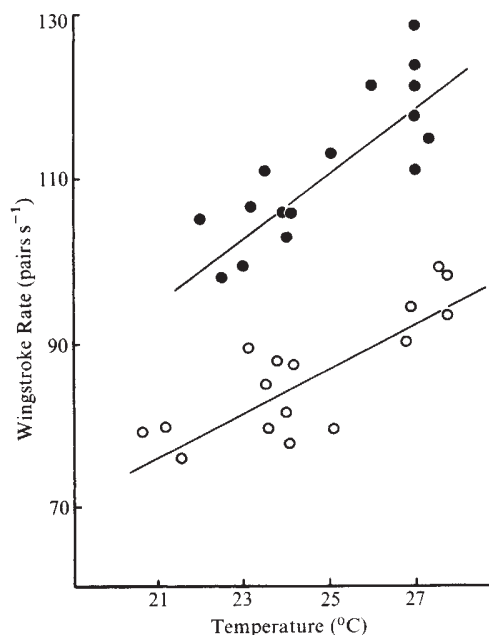
The narrowness of intraspecific variation in insect calling songs is coupled with a narrow range of acoustic parameters that are optimally attractive to conspecific females. Consequently species that call together do not confuse one another. If two species were to use confusingly similar calling songs at the same time and place, rapid evolution towards unambiguously different songs would be expected⁷. Katydids that produce different calling songs generally belong to different species, and calling songs have provided a means of initially recognising species that are morphologically indistinct^{8–10}. A major complication in identifying species by their calling songs is that the rate of the male's sound-producing wingstrokes is a function of ambient temperature¹¹. Differences in rates of sound-producing wingstrokes are often the only important differences among calling songs of similar species^{12,13}. Comparisons must therefore be made between calling songs made by males at the same

ambient temperature, or wingstroke rates must be corrected to compensate for temperature differences. The rates to which females are attracted are also a function of ambient temperature¹².

Neoconocephalus triops is a coneheaded katydid (Copiphorinae) found in southern United States, the Caribbean, and northern South America. In northern peninsular Florida, Walker¹⁴ noted two seasonally distinct populations of adults that differed in the wingstroke rate and continuity of the calling song. Winter males (calling January–April) produced approximately 90 wingstrokes s^{-1} at 25 °C and called for minutes without interruption after initially hesitating about once per second. Summer males (calling July–August) produced approximately 110 wingstrokes per second at 25 °C and continually interrupted their calls at ~1 s intervals (Fig. 1). Although he found no non-overlapping morphological differences, Walker interpreted northern Florida *N. triops* as two species, each with a distinctive univoltine life cycle and calling song. Whitesell¹⁵ refuted this interpretation by periodically sampling field populations and by rearing in outdoor cages. He established that all summer adults are progeny of adults of the previous winter while some winter adults are progeny of adults of the previous summer and some are progeny of adults of the previous winter. Both univoltine and bivoltine life cycles occur in the same deme with summer adults containing only part of the deme's gene pool while winter adults contain all of it. Whitesell also demonstrated that photoperiod was one determinant of whether particular progeny of winter adults became summer or winter adults. Those progeny that made the final molt during photoperiods similar to those of July became reproductively active without delay; shorter photoperiods resulted in adults that were in reproductive diapause—as shown by the silence of the males and the unenlarged ovaries of the females. In outdoor conditions adult diapause ends in January or February but Whitesell discovered that diapausing individuals could be brought to reproductive readiness at any time by exposing them to 15-h photoperiods for a few weeks.

Since northern Florida *N. triops* had proved to be one species rather than two, we sought causes of the unprecedented circumstances of a single species making different calling songs at different seasons.

Fig. 1 Wingstroke rates of north Florida (Alachua Co.), field-collected adults. Regression lines: ●, (summer, July–August) $\hat{y} = 3.99x + 12.13$; ○, (winter, January–April) $\hat{y} = 2.61x + 22.19$, ($x = ^\circ\text{C}$). (Coefficients of determination, r^2 , are 0.71 and 0.65.)



Specimens for experiments were captured near Gainesville, Florida, and caged individually in screen-topped jars with food and water. Two photoperiod chambers, each illuminated by two 15-W fluorescent bulbs and maintained at ambient temperature $\pm 1^\circ\text{C}$ by exhaust fans, provided day lengths of 11 and 15 h—approximately equivalent to the shortest and longest local days. The chambers and control jars were housed in a screened insectary that provided outdoor photoperiods and near-outdoor temperatures. Calling songs were recorded at 38 cm s^{-1} within the first week that an individual began to sing. High-speed cinematography demonstrated that the calling song is produced by alternate long and short closures of the wings¹⁶. Rates for complete cycles of wing movement, consisting of two openings and two closings, were calculated from audiospectrograms.

When reared in 15-h photoperiods (Table 1) progeny of summer adults made songs characteristic of summer adults. When reared in 11-h photoperiods (and kept in diapause for 3 months), they made songs similar to those of winter adults.

The males in Table 1 that produced near-winter songs had been adults for more than 3.5 months when they began to sing. The males producing summer songs had been adults less than one month. Therefore, the differences in calling songs might be attributed to age^{15,17}, to the events of diapause, or to both. To compare the effects of age and diapause, we collected 30 diapausing males from 19–28 September 1972 and kept them in the screened insectary at outdoor temperatures and photoperiods. Successive groups of 10 were moved to a $25 \pm 1^\circ\text{C}$, 15L:9D room on 1 October, 15 November, and 1 January, and their songs were recorded as soon as diapause ended and singing began (Table 2). Even though the earliest group sang when 2 months younger than the latest group, their songs were not significantly different. The proximal causes of seasonally distinct calling songs in *N. triops* are apparently associated with diapause or its initiation or termination.

The ultimate or adaptive causes of the different calling songs are unknown. At one extreme they may simply be by-products of the physiology of diapause. At the other extreme they may be specific, evolved responses—that is, one or both songs may have changed from the ancestral song because of increased reproductive success of individuals that had the changed song(s). If the latter is the case, the critical events of natural selection probably occurred in conditions different from those in northern Florida at present. However, a clue to what may have happened is the simultaneous singing of *Neoconocephalus retusus* and summer *N. triops* in August in north Florida. The

Table 1 Calling songs of males reared from a single collection of nymphs that were progeny of summer adults

	Rearing Photoperiod	
	15-h	11-h
Date of collection	11 Aug 1972	11 Aug 1972
Month of moult to adults	Sept. 1972	Sept. 1972
Date of transfer to recording room*	21 Sept. 1972	1 Jan. 1973
Age of males when recorded	1 month	3.5 months
No. of males recorded	6	4
Song characteristics:		
Phrasing†	summer	winter
Wingstroke rate		
Range	106–116	95–104
Mean	109‡	98‡
S.e.m.	2	2

Specimens at outdoor temperatures and 15- or 11-h photoperiods until transferred to $25 \pm 1^\circ\text{C}$, 15L:9D room prior to tape recording.

* Males from 15-h treatment were singing before transfer; males from 11-h treatment began singing 2 weeks after transfer.

† Summer phrased songs are continuously interrupted at $\sim 1\text{ s}$ intervals. Winter-phrased songs become continuous after initial interruptions.

‡ Means significantly different at $P \leq 0.005$ (Student's *t*-test).

Table 2 Effect of early termination of diapause on wingstroke rates

	a	Group b	c
Date of transfer	1 Oct. 1972	15 Nov. 1972	1 Jan. 1973
Date of first singing	25 Oct.	2 Dec.	4 Jan.
No. of males recorded	4	4	4
Song characteristics:			
Phrasing*	winter	winter	winter
Wingstroke rate			
Range	96–104	92–104	101–103
Mean	100†	99†	102†
S.e.m.	2	2	1

All males collected 19–28 September 1972 and kept at outdoor temperatures and photoperiods until transfer to 25°C , 15L:9D room.

* Winter-phrased songs become continuous after initial interruptions.

† No two means were significantly different at $P = 0.05$ (Student's *t*-test).

calling song of *N. retusus* has wingstroke rates and continuity like that of winter *N. triops*. If summer *N. triops* used the winter song, females of each species would be falsely attracted during their several weeks of overlap.¹⁵

Variation in the morphology of the male genitalia of the leafhopper *Euscelis plebejus* seems to us analogous to that of *N. triops* calling songs¹⁸. What were originally described as two species on the basis of aedeagal differences were shown by E. J. Muller to be seasonal dimorphs controlled by photoperiod. As in *N. triops* no clear cut adaptive significance is evident for a photoperiodically-triggered dimorphism in a reproductively important aspect of the phenotype.

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Climate and reproduction of grizzly bears in Yellowstone National Park

CONTROVERSY surrounds the conflicts between the requirements of human safety and the preservation of grizzly bears (*Ursus arctos horribilis*) in western North America. It has been difficult to separate the effect of factors such as the closure of garbage dumps from that of the climate. It has also proved difficult to relate climatic data to changes in the populations of large mammals. I report here a correlation of climatic change with fluctuations in the sizes of litters of grizzly bears born in Yellowstone National Park, Wyoming, during 1958–1976. The decrease in litter sizes observed since the closure of garbage

dumps seems to be largely a consequence of unfavourable weather during the periods of the final fattening of the mother, winter sleep, birth, lactation and early spring foraging. This study represents one of the few times that the effects of climate have been demonstrated for large omnivorous or carnivorous mammals.

I used modified Lamb indices of winter severity and wetness-dryness¹. In compiling the indices, the signs (plus or minus) of the deviations from the long term temperature and precipitation means recorded at the Yellowstone weather station² were tabulated for 1958–76. Standard deviations for temperature and precipitation were calculated. An extra sign was tabulated for any month with a deviation greater than the standard deviation. These data summed and compared with ratios of grizzly bear cubs to females^{3,4} (litter size). Summer litter sizes were correlated ($r = +0.6589$; $P < 0.01$) with the climate index for the months from October to the following May.

Craighead and Craighead⁵ observed that periods of 'wintry' weather in October seemed to trigger grizzly bears to select dens and begin their winter sleep. October may be an important time relative to reproductive success in the Yellowstone area. Rogers⁶ found that the success of reproduction in black bears (*Ursus americanus*) in Minnesota was strongly influenced by food availability. Mealey⁷ reported that Yellowstone grizzlies eat primarily grass, sedges and the nuts of White bark pine (*Pinus albicaulis*) in September and October. The cones of White bark pine are shed in September and October. 'Wintry' weather undoubtedly affects the availability of grass and nuts to the bears. October weather may also be important because it determines whether the female bear will continue adding to her energy reserves or begin using them. Rogers⁶ speculates that black bears take stock of their stored nutrient reserves in the autumn and prevent implantation of fertilised ova during November or December, in years when the nutrient stores were too low.

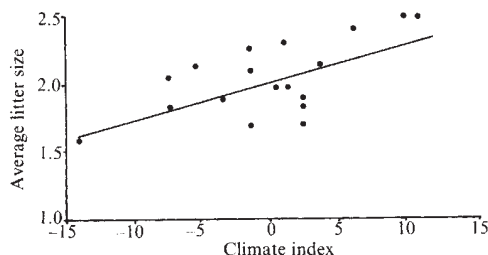


Fig. 1 Plot of mean sizes of grizzly bear litters in Yellowstone National Park against a climate index for 8-month annual sequences from October to May during 1958–76. $\hat{y} = 2.052 + 0.0289x$; $r = 0.6589$; $P < 0.01$.

The bears emerge from their dens in late March and early April, and females with their cubs are the last to leave^{5,8}. The snowpack at the 2,400 m elevation in Yellowstone usually builds up until April, with the spring thaw coming in May and early June. Early spring foraging is undoubtedly important to the lactating female grizzly bear and the survival of her cubs, and the importance of carrion to them between March and May in this park has been reported^{5,9}. An index of relative availability of carrion for 1966–76 was computed from my data and those from the National Park Service¹⁰. This index was combined with the climatic index, increasing the correlation ($+0.7595$; $P < 0.01$) between the index and litter size.

The pattern of weather during 11 yr when litter sizes were greater than two differed from that of the 7 yr when they were less than two. When litters were large, Octobers were warmer and drier, implying an extended feeding period during the autumn; winters were warmer and had more snow, reducing the energy needs of the bears during winter; the Aprils were warmer and Mays warmer and drier, improving the availability of food in the early spring. These factors seemed to improve the ability of cubs to survive into the summer when the litter size measurements were obtained.

The closure of garbage dumps in Yellowstone during 1970 and 1971 accentuated the controversy concerning the management of bears in the park¹¹. The ratio of cubs to females averaged 1.8 after closure (1972–76) compared with 2.2 ($P < 0.001$) during 1959–69. But when I corrected the ratios for 1972–76 and 1966–70 for the influence of climate and carrion, I found no differences ($P < 0.4$) between the mean ratios for the two periods. Uncorrected, they differ with a probability of < 0.1 . The climatic index suggests that conditions have been relatively unfavourable for grizzly bears to reproduce during 3 of the 5 yr since the closure of the garbage dumps. Seven of the 11 yr before closure were relatively favourable. The human population (and thus garbage production) of Yellowstone is highest in July and August. Thus food availability at the garbage dumps was not high during the spring and fall periods which seem to be critical.

My results suggest that the recent depression in the reproductive rate of grizzly bears has been due to influences related to climate, and the closure of garbage dumps has had little effect. The effect of climate on the survival of the yearling age class, however, has not been defined.

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Initiation of UV mutagenesis in *Saccharomyces cerevisiae*

STUDIES of UV mutagenesis in *RAD*⁺ and *rad1-1* (excision defective) strains of yeast have shown that the majority of forward mutations induced in *RAD*⁺ occur before the first cell division following UV exposure, whereas in *rad1-1* the first mutations only appear after the cell has divided and DNA replication has presumably occurred^{1,2}. These data have been interpreted as showing that error-prone repair in both *RAD*⁺ and *rad1-1* is initiated by the production of a single-stranded gap opposite a pyrimidine dimer in the complementary DNA strand. However, although this structure may be the same in both strains, it probably arises in entirely different ways. In *RAD*⁺ it can, in theory, be formed before DNA synthesis occurs provided two dimers are induced in complementary DNA strands sufficiently close together for excision of one to uncover the other (compare refs 3 and 4). In *rad1-1*, which lacks excision, this cannot occur and, by analogy with *E. coli*, it is believed that only when dimers pass through DNA synthesis and daughter strand gaps are formed⁵, can error-prone repair be initiated. If these ideas are correct two predictions can be made concerning the behaviour of reverting auxotrophs in UV experiments with *RAD*⁺ and *rad1-1*. Here we test these two predictions. The results support the dimer/gap model.

Prediction (1). Since DNA synthesis is thought not to be necessary for dimer-dependent mutagenesis in *RAD*⁺, neither the yield of reversions nor their photoreversibility should be affected if cell division and DNA synthesis after exposure to UV are restricted. In contrast, if dimer-induced reversions in *rad1-1* cannot be produced unless DNA synthesis takes place, any reversions obtained on a medium which prevents divisions in the irradiated population should be non-photoreversible. Once cell division and DNA synthesis are permitted, the yield of mutations should be enhanced and the extra mutants should be largely photoreversible. Provided the reverting auxotroph is not leaky, it is easy to test this since growth after UV exposure can often be restricted to less than one division per cell on average by using unsupplemented minimal medium. Alternatively, any number of divisions can be permitted by adding predetermined trace levels of the required supplement to the medium.

Prediction (2). The kinetics of mutation induction by UV should also differ in the two strains. Two dimers must cooperate in *RAD*⁺ before excision can create the dimer/gap structure and mutation induction kinetics should reflect this in displaying two-hit kinetics. Only one dimer is needed to produce a daughter strand gap in *rad1-1* and one-hit mutant induction kinetics should be found.

The alleles used for the reversion experiments were two ochre mutants *arg4-17* and *lys1-1*, provided in *RAD*⁺ and *rad1-1* backgrounds by A. P. James. We have shown that at least 98% of the revertants obtained after UV exposure are probably true revertants and are not attributable to the suppression of these ochre alleles. Cells were grown for 48 h in yeast extract peptone dextrose (YEPD) medium before washing in water and preparing a suspension of cells containing about 2×10^7 ml⁻¹. Standard methods were used for inactivation, photoreactivation and the determination of survival and induced mutations. UV doses for all experiments other than the determination of dose-effect curves were 30 erg mm⁻² for *rad1-1* and 300 erg mm⁻² for *RAD*⁺. For photoreactivation, samples were exposed for 30 min to four warm white 15-W fluorescent tubes at a distance of 10 cm. Cells were plated on yeast extract agar to determine viabilities and reversions were scored either on minimal medium which allowed 0.6 divisions per cell to occur, or on minimal medium supplemented with arginine or lysine at 1 µg ml⁻¹. Approximately six cell divisions occur at this level of supplementation.

Table 1 gives the results of duplicate experiments on the effects of trace supplements on mutation yield and photoreactivation in *arg4-17* and *lys1-1* in the *RAD*⁺ and *rad1-1* backgrounds. The frequencies of both arginine and lysine revertants are increased markedly when *rad1-1* cells are encouraged to complete a few divisions on the mutation plates. The factor of increase for mutations recovered on minimal medium plus supplement compared with minimal medium alone ranges from

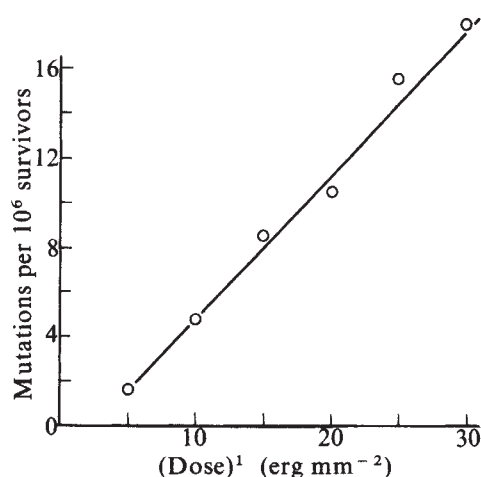


Fig. 1 Induction of reversions in *rad1-1* by UV.

4 for *arg4-17* reversions to nearly 10 for *lys1-1* reversions. There is no consistent action of supplemented medium on the yield of either type of reversion in *RAD*⁺ strains.

In these experiments the irradiated cells suffer stepdown conditions when plated for reversions. Since this might itself affect the mutant yield quite apart from the number of divisions, cells were also plated on minimal medium supplemented with levels of YEPD ranging from 0.1% to 1.0%, the upper limit for the detection of revertants against the background growth. 0.1% YEPD, which is approximately equivalent to 1.0 µg ml⁻¹ arginine in terms of the divisions it allows, produced the same number of reversions as 1.0 µg ml⁻¹ arginine in both strains. Furthermore even an increase in YEPD concentration to 1.0% failed to affect the mutational yield in *RAD*⁺ and increased it by only a factor of 1.5 in *rad1-1* in spite of the additional divisions which took place. Stepdown conditions therefore seem to be of little importance in the present context.

Table 1 also shows clearly that the additional mutations, which appear in *rad1-1* when traces of supplements are added to the mutation medium, are strongly photoreversible. Roughly to 50–70% of the extra *arg*⁺ revertants and as many as 80% of the extra *lys*⁺ revertants are photorepairable and therefore dimer-associated in origin. This contrasts with a much lower level of photoreactivation when samples from the same treated suspension are plated on minimal medium alone. Trace supplementation has no effect on the extent of photoreactivation in *RAD*⁺ material.

The response of *arg4-17* to different doses of UV is shown in Figs 1 (*rad1-1*) and 2 (*RAD*⁺). In both cases traces of arginine were added to the minimal plates used for scoring revertants.

Table 1 Effect of traces of arginine and lysine on UV-induced mutation and photoreactivation of *arg4-17* and *lys1-1* in *rad*⁺ and *rad1-1* backgrounds

Strain:	<i>rad1-1 arg4-17</i>		<i>rad1-1 lys1-1</i>		<i>rad</i> ⁺ <i>arg4-17</i>		<i>rad</i> ⁺ <i>lys1-1</i>	
Survival	I	II	I	II	I	II	I	II
Untreated	100%(221.7)	100%(65.3)	100%(127.0)	100%(188.6)	100%(200)	100%(111.6)	100%(151.4)	100%(229.7)
UV dark	46.3%(205.2)	58.6%(76.5)	34.0%(86.4)	42.2%(159.3)	70.1%(280.5)	71.1%(158.6)	66.4%(201)	67.4%(309.7)
UV + PR	83.4%(184.8)	118.6%(77.5)	93.1%(118.3)	95.8%(18.7)	97.0%(193.9)	112.8%(125.9)	113%(171.3)	100%(229.7)
Mutations (Minimal medium) × 10 ⁻⁶ survivors								
Untreated	0.01(0.06)	0.04(0.05)	0.07(0.17)	0.18(0.67)	0.02(0.67)	0.03(0.067)	0.07(0.2)	0.04(0.2)
UV dark	4.6(9.75)	7.57(5.83)	3.21(2.77)*	1.3(2.07)*	33.5(93.67)	41.01(65.2)	27.2(54.3)	20.5(63.6)
UV + PR	3.9(14.30)	5.48(8.50)	1.48(3.5)*	1.04(3.76)*	22.3(86.6)	23.8(60.0)	12.5(42.5)	9.37(43.1)
Mutations (+ δ supplement) × 10 ⁻⁶ survivors								
Untreated	0.75(0.33)	0.31(0.40)	0.16(0.4)	0.21(0.78)	0.03(0.13)	0.48(1.07)	0.02(0.07)	0.15(0.7)
UV dark	18.7(39.3)	21.04(16.20)	13.9(12.0)	11.93(19.0)	35.3(98.87)	56.9(90.4)	24.7(49.3)	21.2(65.7)
UV + PR	11.1(41.1)	10.20(15.8)	3.4(8.0)	3.35(12.1)	22.9(89.07)	29.7(74.9)	12.8(43.6)	8.8(40.4)

Figures in parentheses represent the average number of colonies counted per plate. Except where indicated 10 plates were used for survival estimates and between 5 and 10 plates for estimates of mutations. PR, photoreactivation.

* Based on 26–30 plates.

The addition is not important for the *RAD*⁺ strain but it is probably essential for the *rad1-1* strain if dimer-related mutagenesis is to be observed and the kinetics determined. The results shown are the averages of two independent experiments in each case. The replicates were in good agreement. The lines have been drawn by eye, but even without them it is clear that in the *RAD*⁺ strain *arg*⁺ mutants are produced in a two-hit or near two-hit fashion while in *rad1-1* one-hit kinetics are approximated.

These results are completely consistent with a model of mutagenesis in which the dimer/gap structure is the initiator of mutagenesis in yeast. It is clear that in *RAD*⁺ the initiation of UV mutagenesis is not dependent on DNA replication and that a large proportion of the mutants produced are photoreactivable and thus of dimer-related origin. In contrast, replication seems to be indispensable if dimers are to be mutagenic in *rad1-1*. The few mutants obtained on minimal medium in this strain may include some which are dimer-related because photorepair is not completely abolished. However, since even on minimal medium some cells still accomplish a division, these can be accounted for. Most of the few mutants found on minimal medium are not photoreactivable and presumably arise from non-dimer sources. Furthermore it can be calculated that this number is not augmented when replication occurs. The photoproducts concerned, therefore, are presumably able to generate mutations without the need for DNA replication.

The data on the kinetics of mutant induction also confirm our prediction that, whereas two dimers must collaborate to produce the initiating signal for error-prone repair in *RAD*⁺ yeast, only one dimer is required in *rad1-1*.

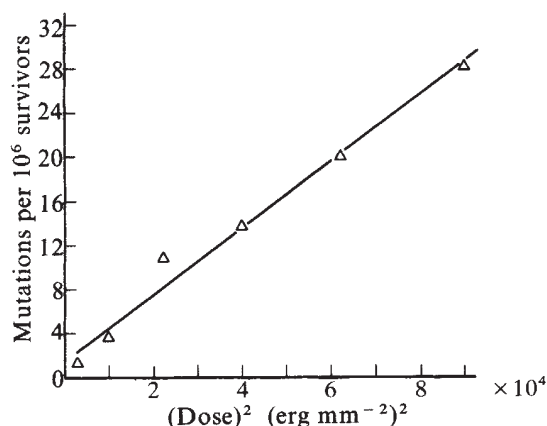


Fig. 2 Induction of reversions in *RAD*⁺ by UV.

In addition to providing support for the dimer/gap model, these results suggest a simple explanation for some apparent disagreements between other workers who have studied reversion after UV in *RAD*⁺ and excision defective yeast. Resnick pointed out first of all that certain auxotrophs failed to respond to photoreactivation in excision defective yeast and suggested that a difference existed between base-pair substitutions and frameshift mutants in this respect⁶. Lawrence *et al.*⁷ showed that an iso-1-cytochrome *c*, base-pair substitution mutant which reverted readily with UV in an excision deficient strain displayed marked photoreactivation of reversion. Forward mutations at a variety of loci in *rad1-1* yeast are also subject to photorepair (B. J. K. & A. P. James, in preparation). It now seems likely that these observations can readily be reconciled. Lawrence *et al.*⁷ allowed several cell divisions after UV for 'expression' of the revertants, and forward mutations require divisions and as a matter of course. Resnick on the other hand plated on minimal medium. The difference in response to dimers between base-pair substitution mutations and frameshift mutations he suggested may rather be a question of slight leakiness or otherwise of the strains he used. There is no

other evidence that pyrimidine dimers induce or revert frameshift mutations preferentially^{8,9}.

Note added in proof: More extensive data show that *arg*⁺ reversions accumulate in proportion to dose^{1.8 to 1.9} in *RAD*⁺ and in proportion to the 1.2 nd power of one dose in *rad1-1*.

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Saccharomyces cerevisiae cell cycle mutant *cdc9* is defective in DNA ligase

IN the yeast *Saccharomyces cerevisiae* a number of mutants potentially defective in DNA replication have been isolated¹⁻³. However, the only defective gene product so far identified in any of these mutants is that in *cdc21* which is defective in thymidylate synthetase^{4,5}. We report here that *cdc9-1*, isolated by Hartwell as a conditional cell cycle mutant⁶, is defective in a DNA ligase involved in both replication and repair of DNA. A mutant with similar properties has recently been described in *Schizosaccharomyces pombe*⁷.

At the restrictive temperature *cdc9* arrests in medial nuclear division after an apparently normal round of DNA synthesis⁶. Its defective gene product is active in an event normally completed by the end of the S phase⁶ and it is dependent on concomitant DNA synthesis to complete its function in the cell cycle⁸. We have recently examined the pattern of DNA synthesis in wild-type *S. cerevisiae* in alkaline sucrose gradients after pulse-labelling of DNA⁹. Analysis of *cdc9* by this technique has shown that the DNA synthesised at the restrictive temperature (37 °C) is predominantly in the form of small fragments, whereas the parental (pre-labelled) DNA remains full sized (Fig. 1). The defect is temperature dependent, there being no accumulation of these fragments at the permissive temperature (23 °C) (Fig. 1b). The fragments in the peak fraction are approximately 130,000 daltons in size (see Fig. 1), equivalent to 400 nucleotides, which is similar to that of Okazaki pieces in other eukaryotes¹⁰. Approximately 80% of the nascent DNA is present as small pieces after labelling at 37 °C, which implies that these molecules are derived from both strands of the DNA replication growing point.

The mutant *cdc9* is not only defective in DNA replication but also in the repair of DNA damaged by UV irradiation. At the permissive temperature *cdc9* is not markedly more sensitive than the wild type, but incubation after irradiation for 1 h at 37 °C (a treatment which all non-irradiated cells survive) results in a significant increase in sensitivity to UV irradiation (Fig. 2).

The dual role of the *cdc9* gene product in both DNA replication and repair suggests that *cdc9* may be defective in an enzyme such as DNA ligase, which is thought to be involved in both of these processes. We have therefore compared the DNA ligase activity of mutant and wild-type extracts by a procedure

that detects the conversion of 'open' DNA circles containing a single ligatable nick to a closed circular product. This product is distinguished from the substrate by its more rapid electrophoretic migration in agarose gels containing ethidium bromide. DNA ligase activity is readily observed in crude extracts of wild-type strains at both permissive and restrictive temperatures (Fig. 3). However, *cdc9* has no detectable activity at either temperature, even when the cells are grown at the permissive temperature.

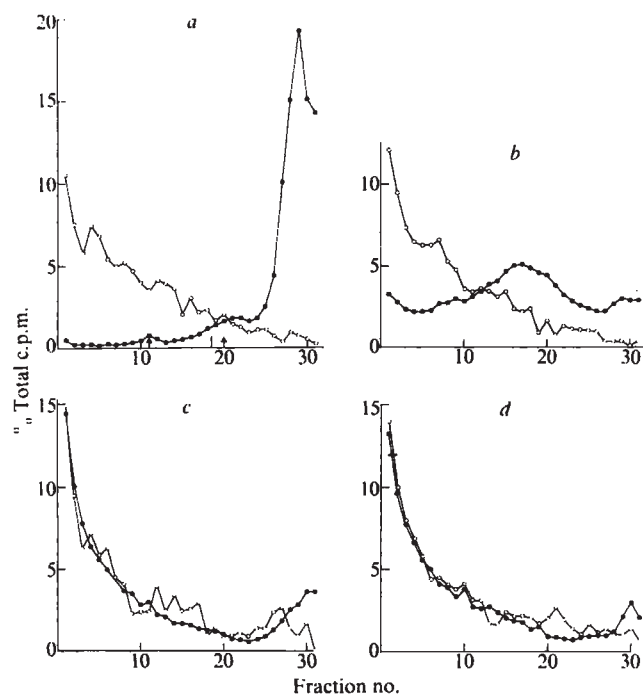


Fig. 1 Alkaline sucrose gradient analysis of pulse-labelled DNA from *cdc9*, a revertant of *cdc9* and the wild type. The gradients in *a*, *b* and *d* were carried out using cytoplasmic petite mutants containing no mitochondrial DNA. Mid-log phase cells grown in YE_{PD}³ and pre-labelled for some eight generations with $0.06 \mu\text{Ci ml}^{-1}$ ^{14}C -uracil were resuspended in 1 ml fresh medium at 7×10^7 cells ml^{-1} . After 5 min incubation at 25 °C, cells were ready for pulse-labelling with $70 \mu\text{Ci ml}^{-1}$ $[8\text{-}^3\text{H}]\text{adenine}$. Pulses were terminated by the addition of 1 ml 3% toluene, 95% ethanol and 10 mM EDTA⁹. The cells were washed by centrifugation and treated with zymolase to digest cell walls. After centrifugation the pellet was resuspended in $50 \mu\text{l}$ 10 mM EDTA and cells were lysed by the addition of $200 \mu\text{l}$ 8% sarkosyl and 0.5 M NaOH. Linear gradients were prepared from 5 and 20% (w/v) sucrose made up in 0.5 M NaCl, 0.25 M NaOH, 1 mM EDTA, 0.2% sarkosyl and $1 \mu\text{g ml}^{-1}$ sonicated calf thymus DNA. Fractions were collected in tubes, made 0.5 M with NaOH and incubated for 24 h at 37 °C. Macromolecules were precipitated with trichloroacetic acid⁹, and radioactivity determined using a toluene-based scintillant. Centrifugation was at 19,000 r.p.m. for 16 h at 20 °C in a Beckman SW40 rotor. Sedimentation is from right to left. Arrows on *a* indicate the position to which the DNA of phages λ and T4 sedimented. Using these markers in the equation of Levin and Hutchinson¹⁴ a value for coefficient α of 0.36 was calculated and this was used to determine molecular weights in these gradients. *a*, *cdc9*, 10-min pre-incubation at 37 °C, followed by a 30-min pulse at 37 °C (total ^{14}C -label: 557 c.p.m.; total ^3H -label: 9,813 c.p.m.); *b*, *cdc9*, 30-min pulse at 25 °C (total ^{14}C -label: 550 c.p.m.; total ^3H -label: 15,269 c.p.m.); *c*, revertant of *cdc9* capable of growth at 37 °C 10-min pre-incubation at 37 °C, followed by a 30-min pulse at 37 °C (total ^{14}C -label: 321 c.p.m.; total ^3H -label: 15,275 c.p.m.). Mitochondrial DNA appears between fractions 24 and 28 (ref. 9); *d*, wild-type parent of *cdc9* 10-min pre-incubation at 37 °C, followed by a 30-min pulse at 37 °C (total ^{14}C -label: 291 c.p.m.; total ^3H -label: 14,990 c.p.m.). ○, ^{14}C -label; ●, ^3H -label.

Although *cdc9* has a pleiotropic phenotype, evidence suggests that its various defects are the result of a single nuclear mutation. First, data from 15 tetrads, isolated after crossing with a suitable wild type (D273-11a; ref. 3), indicated a 2:2 segregation for sensitivity to growth at 37 °C, thus confirming¹¹ that a single mutation is responsible for the temperature-sensitive phenotype. In all 15 tetrads UV sensitivity co-segregated with the failure to grow at 37 °C, and in the three tetrads tested these two characters co-segregated with the absence of ligase activity (Fig. 4). The products of one tetrad were also analysed by alkaline sucrose gradient sedimentation and the failure to join Okazaki pieces at 37 °C co-segregated with these other properties. Second, a revertant of *cdc9-1* able to grow at 37 °C (but otherwise identical to *cdc9-1* in genotype) is capable of joining nascent DNA at the restrictive temperature (Fig. 1), is less sensitive to UV irradiation (Fig. 2) and has nearly normal levels of DNA ligase activity at both permissive and restrictive temperatures (Fig. 3).

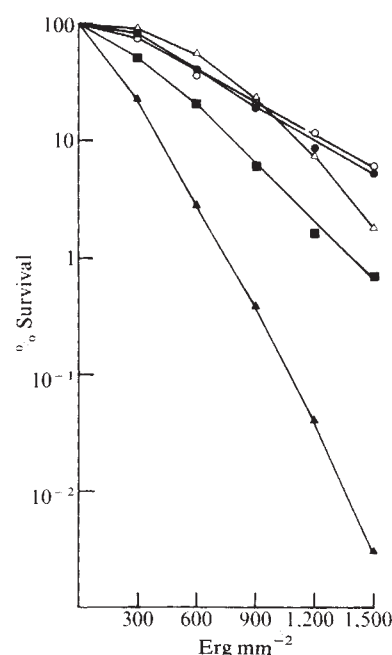
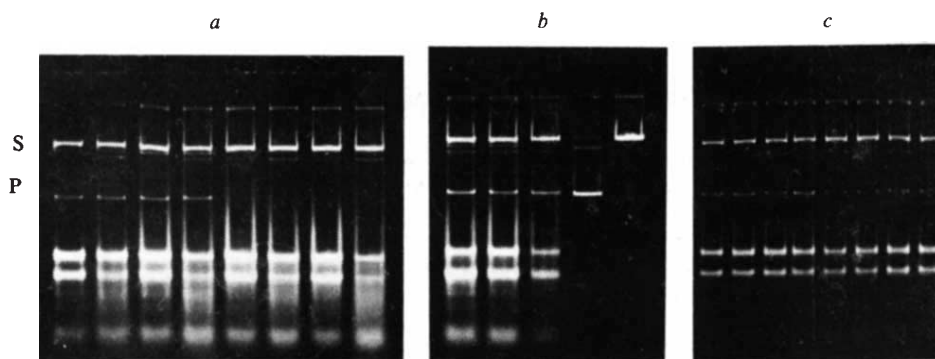


Fig. 2 Response to ultraviolet light of *cdc9*, a revertant of *cdc9* and the wild type. Cells grown at 25 °C to mid-log phase were diluted, spread on the surface of YE_{PD} agar and irradiated with the required dose. After irradiation plates were either placed at 37 °C for 1 h and then transferred to 25 °C for further incubation or were incubated solely at 25 °C. Plates were scored after at least 7 d incubation. ○, Wild type, 25 °C; ●, wild type, 1 h at 37 °C; Δ, *cdc9*, 25 °C; ▲, *cdc9*, 1 h at 37 °C; ■, revertant of *cdc9*, 1 h at 37 °C.

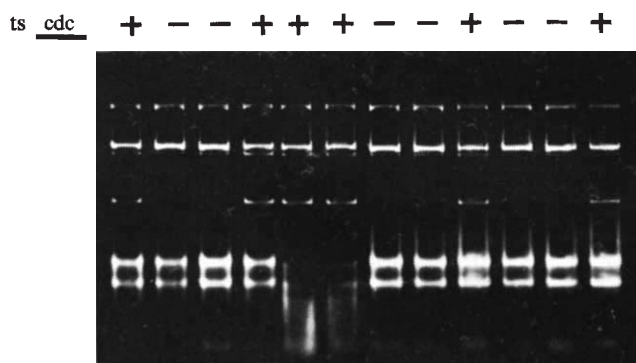
The phenotype of *cdc9* and its similarity to that of the well characterised *Escherichia coli* ligase mutant¹² suggest that *cdc9* is defective in DNA ligase. However, in the absence of detectable *in vitro* activity we cannot yet conclude that the ligase itself is temperature sensitive. The existence of a mutant should help elucidate the physiological role of DNA ligases in eukaryotic cells. For example, preliminary evidence indicates that the ligase defective in *cdc9* acts on mitochondrial DNA and is also involved in repair of damage due to methyl methane sulphonate. Furthermore, the cell cycle properties of *cdc9* suggest that no essential DNA metabolism requiring ligation occurs throughout G2 or mitosis, though it is possible that, as in mammalian cells¹³, *S. cerevisiae* possesses two DNA ligases, the second having

Fig. 3 DNA ligase activity in crude extracts of *cdc9*, a revertant of *cdc9* and the wild type. The substrate consisting of 'open' DNA circles of the plasmid pBR322 (ref. 15) was prepared as follows: closed circular plasmid DNA was purified from chloramphenicol-treated cells of *E. coli* by banding twice in CsCl gradients containing ethidium bromide. The ethidium was removed from the DNA by five extractions with butan-1-ol saturated with TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.5); DNA was precipitated at -20°C using 70%



ethanol, collected by centrifugation and dissolved in TE buffer. DNA prepared by this method consisted entirely of closed circular supercoiled monomers (approximately 95%) and dimers of pBR322 (form I). It was converted to an open form containing a single nick in one strand (form II, the DNA ligase substrate) by digestion with DNase I (Sigma) in the presence of ethidium bromide¹⁶. Ligation of such DNA creates a population of closed circular molecules with a varying degree of supercoiling¹⁷ (form IV). In the presence of ethidium bromide this population of molecules migrates as a single band during electrophoresis in agarose gels (b, track 4), with a greatly increased velocity over that of open circles (b, track 5). Further detail concerning the kinetics and quantitation of this assay will be published elsewhere. Each assay was carried out by the addition of 1 μl enzyme extract to 11 μl DNA substrate at 12.25 $\mu\text{g ml}^{-1}$ in 66 mM Tris-HCl (pH 7.5), 10 mM MgCl_2 , 1 mM EDTA, 1 mM DTT, 1 mM ATP and 100 $\mu\text{g ml}^{-1}$ bovine serum albumin. The reaction was stopped by addition of 2.2 μl 15% Ficoll, 100 mM EDTA and transfer to 0°C . The DNA was then extracted once with 25 μl phenol/cresol mixture saturated with water, and 10 μl of the aqueous phase was loaded on to 6×2 mm slots in a vertical slab gel electrophoresis apparatus (1% agarose, made up in $1 \times \text{E}$ buffer¹⁸ containing 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide). This was run at 5 V cm^{-1} for approximately 90 min. The gel was then removed and photographed under UV light¹⁹. The following nucleic acid species can be seen in the gels from top to bottom: form II dimers of pBR322; form II monomers of pBR322 (marked S, the main DNA ligase substrate); a series of faint bands which run slightly faster than the latter (these include linear—form III—monomers of pBR322 created by nuclease action, and form IV dimers of pBR322); form IV monomers of pBR322 (marked P, the main DNA ligase product); 18S and 28S rRNA; and finally 4S and 5S RNA. The RNA species may be used as an indication of the quantity of crude extract added to the substrate mixture. a, Comparison of DNA ligase activity of *cdc9-1* and wild type. *cdc9-1* and wild type were grown at 23°C in YEPD (supplemented with 75 $\mu\text{g ml}^{-1}$ adenine and uracil) to mid-log phase, at which point each culture was diluted twofold and split, one half being left at 23°C , whereas the other was shifted to 37°C . After 90 min, the four cultures were rapidly cooled to 0°C , the cells collected by centrifugation, and crude extracts prepared as described previously⁷. The extracts were adjusted to $A_{260} = 46$ (approximately 4.5 mg ml^{-1} protein) and each extract was assayed for DNA ligase activity at 23°C and 37°C (10 min incubation). Track 1: assay 3°C of wild type grown at 23°C ; track 2: assay at 37°C of wild type grown at 23°C ; track 3: assay at 23°C of wild type shifted to 37°C ; track 4: assay at 37°C of wild type shifted to 37°C ; tracks 5–8: as 1–4 but with *cdc9-1*. b, Wild type extracts still active when mixed with those of *cdc9-1*. Mid-log phase cultures of wild type (in this instance, spore 9d from the cross between *cdc9-1* and D273-11a) and *cdc9-1* (spore 9c) were shifted to 37°C for 90 min before collecting and preparing crude extracts. Both extracts were adjusted to $A_{260} = 46$; incubations were at 37°C for 15 min. Track 1: wild type; track 2: 1:1 mixture of extracts from wild type and *cdc9*; track 3: 1:1 mixture of extract from wild type and extract buffer; track 4: assay of phage T4 DNA ligase; track 5: substrate alone, incubated as above. c, Comparison of DNA ligase activity of wild type and a revertant of *cdc9-1*. This was carried out as described for a, except that the extracts were adjusted to $A_{260} = 43$ and the incubations were for 15 min. Track 1: assay at 23°C of wild type grown at 23°C ; track 2: assay at 37°C of wild type grown at 23°C ; track 3: assay at 23°C of wild type shifted to 37°C ; track 4: assay at 37°C of wild type shifted to 37°C ; tracks 5–8: as 1–4 but with the revertant of *cdc9*.

Fig. 4 Co-segregation of DNA ligase deficiency and temperature-sensitive *cdc* phenotype. Crude extracts from spore clones of three complete tetrads (4a–d, 6a–d, 9a–d, derived from the cross with D273-11a) prepared from cells grown at 23°C (as in Fig. 3). Each extract was adjusted to $A_{260} = 60$ and assayed for DNA ligase activity at 23°C (15 min incubation) as described previously. In this experiment the substrate of open pBR322 circles was at 22 $\mu\text{g ml}^{-1}$. The very low level of form IV DNA (ligase product) apparently formed by the *cdc9* extracts is probably not due to DNA ligase. The substrate used in this experiment, unlike that used for Fig. 3, contained a low proportion of unopened supercoiled DNA (form I). The action of a DNA-untwisting enzyme²⁰ on such molecules relaxes them to give a product which migrates at the same velocity as the DNA ligase product.



specific functions in these phases of the cell cycle. Further analysis of *cdc9* mutants should help in resolving these and other questions.

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Evidence for a cytoplasmic melatonin receptor

MELATONIN, a secretion of the pineal gland, has profound inhibitory effects on the development and maturation of gonadal organs in mammals¹. Pinealectomy of the rat or hamster results in premature enlargement of the uterus and ovary^{2,3} in females and gonadal organs in males^{4,5}, while melatonin administration reverses the effects of pinealectomy and suppresses gonadal growth and maturation in these species¹. The physiological site and mechanism of action are uncertain. The hormone seems to act both in the central nervous system and at peripheral sites; previous studies have demonstrated inhibition of luteinising hormone (LH) and follicle stimulating hormone (FSH) secretion by the pituitary^{5,6} as well as inhibition of oestrogen and progesterone synthesis by ovarian tissue slices^{7,8}. Since other hormones are known to exert their effects through binding to receptor proteins in target organs, we have examined melatonin binding in hamster and rat tissues and human ovaries, and here report evidence for the presence of a specific melatonin receptor in the supernatant fraction of several organs.

Tissues were obtained from female Syrian golden hamsters (aged 60–90 d, from the NIH Animal Resources Branch) and from Sprague-Dawley female rats (60–90 d, Hazleton Laboratories); and from a 42-yr-old female patient with metastatic breast cancer who underwent a therapeutic oophorectomy (the ovaries of this patient were normal on histological examination).

Specific binding of ³H-melatonin, defined by Scatchard analysis of the difference in ³H-melatonin bound in the absence and presence of vast excess of unlabelled melatonin, was detected in the 105,000g supernatant from ovarian tissue of the hamster, rat, and human (Fig. 1). Maximum specific bind-

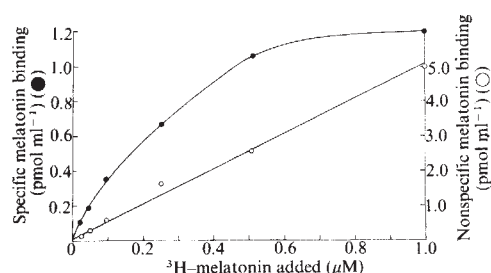


Fig. 1 Tissues were obtained from female golden Syrian hamsters (NIH) and Sprague-Dawley female rats (Hazleton). Animals were killed by cervical dislocation. Tissues were either used immediately after sacrifice or were frozen in liquid nitrogen and stored at -70°C . Frozen tissues were pulverised. Fresh and pulverised frozen tissues were homogenised at 4°C in 0.01 M Tris-HCl, pH 7.7, with three 20-s bursts of a Polytron PT 10-35 sonicator (Brinkman). The homogenate was spun at 106,000g for 1 h at 5°C , and the supernatant removed with a Pasteur pipette (avoiding contamination by the floating lipid layer). Binding was measured by incubating 420 μl of supernatant containing ~ 2 mg protein with ³H-melatonin (NEN, 35.3 Ci mmol⁻¹) at concentrations of 1×10^{-9} to 1×10^{-6} M. 'Nonspecific' binding was determined by addition of the specified concentration of ³H-melatonin plus 10^{-4} M nonradioactive melatonin. After the incubation period, the unbound melatonin was adsorbed by the addition of 0.8 ml of dextran-coated charcoal (5% activated charcoal; 2.5% high molecular weight dextran in 0.01 M Tris-HCl, pH 7.7). The tubes, now containing dextran-coated charcoal, were vortex-mixed for 10 s and incubated for an additional 10 min at 4°C . The charcoal was then pelleted by centrifugation at 2,000g for 30 min. 0.8 ml of the supernatant was added to scintillation vials and counted. Protein concentrations were measured by the method of Lowry *et al.*¹⁵, using bovine serum albumin fraction V as the standard.

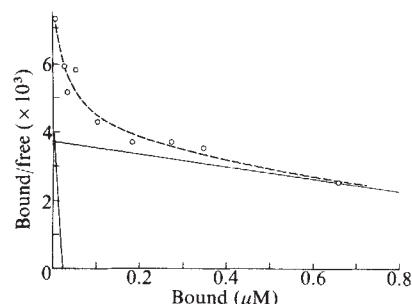


Fig. 2 Scatchard analysis of ³H-melatonin binding in hamster ovarian cytosol (2.7 mg ml^{-1}) which was prepared and incubated under the standard conditions described in Fig. 1. Specific binding was defined as the difference between counts bound in the absence and presence of vast excess nonradioactive melatonin and was subjected to Scatchard analysis⁹. Solid lines represent the computer-fitted components of a curve composed of two classes of binding sites (see text). Assuming two classes of sites, the following constants were derived:

	High-affinity site	Low-affinity site
K_D	$6.3 \times 10^{-9} \text{ M}$	$5.5 \times 10^{-7} \text{ M}$
Slope of regression line	$-3.6 \times 10^{-5} \text{ M}$	$-3.7 \times 10^{-6} \text{ M}$
r -value for regression line	0.95	0.88
No. of binding sites (fmol per mg protein)	52	418.7

ing, which was achieved in 3 h and was stable thereafter for an additional 9 h, was heat labile (complete loss of specific binding at 70°C for 10 min), and was diminished by concentrations of Na^+ , K^+ , Mg^{2+} , or Ca^{2+} in excess of 0.01 M. Specific binding was saturable at 1×10^{-6} M ³H-melatonin, in contrast to nonspecific binding. Scatchard analysis⁹ revealed a curvilinear plot (Fig. 2). Similar results were obtained in multiple experiments. Computer-assisted analysis of data from four such experiments was performed using a nonlinear, least squares, curve-fitting program⁹. A mathematical model utilising two classes of binding sites provided a close empirical approximation of the data, and yielded dissociation constants of 6.3×10^{-9} M for the high affinity, low capacity sites, and 5.5×10^{-7} M for the low affinity, high capacity sites (see Fig. 2 legend). The possibility of negative cooperativity or the presence of more than two classes of binding sites could not be excluded by these results.

³H-melatonin binding was detected in ovarian tissue from the hamster, rat, and human, as well as testis, uterus, skin, liver, and eye of the rodent species (Table 1). Binding site concen-

Table 1 Tissue specificity of melatonin binding

Organ	Species	No. assays (Positive/Total)	Melatonin bound (fmol per mg protein)
Ovary	Hamster	30/30	250–656
	Rat	20/26	298–444
	Human	1/1	330
Uterus	Hamster	30/30	218–565
	Rat	12/18	211–413
Liver	Hamster	12/12	156–1004
	Rat	1/1	292
Eye	Hamster	3/3	44–72
Thyroid	Hamster	1/2	251
Brain	Hamster	0/3	—
Heart			
Lung			
Muscle	Rat	0/3	—
Spleen			
Gastrointestinal tract			
Adrenal Gland	Hamster	0/2	—
Testes			
	Hamster	2/2	270

trations in positive organs estimated from the Scatchard plots varied from 250–656 fmol per mg protein in hamster ovary, to 156–1,004 fmol per mg protein in rat uterus. Other tissues, with the exception of liver and eye, did not contain perceptible ^3H -melatonin binding in these experiments. In particular, no binding was detected in whole brain homogenates, although the possibility of binding to pituitary, pineal, or hypothalamus was not conclusively ruled out by these results.

Binding specificity for ligand structure was examined using various indole amines and analogues (Fig. 3). ^3H -melatonin binding was readily inhibited by competition with unlabelled melatonin and 6-fluoromelatonin, but was less well competed, in decreasing order of affinity, by 5-methoxyindole > 5-hydroxytryptamine > 5-methoxytryptamine. Tryptophan and 4,6-difluoromelatonin had no effect on ^3H -melatonin binding. Neither was there discernible competition for binding by other neurogenic amines, including histamine, dopamine, or epinephrine, all of which bind to distinct membrane-bound receptor proteins.

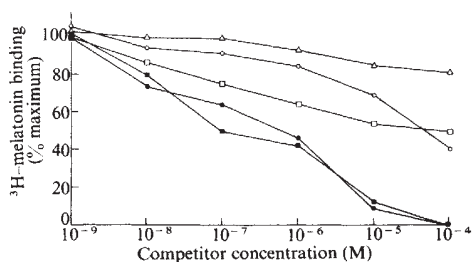


Fig. 3 Competition of indolamines for ^3H -melatonin binding sites. Increasing concentrations of melatonin and other indolamines were added to tubes containing $1 \times 10^{-7} \text{ M}$ ^3H -melatonin and incubated for 4 h with hamster ovarian supernatant as described in Fig. 1. After incubation, the unbound ^3H -melatonin was adsorbed by addition of dextran-coated charcoal as described. Δ , 5-methoxytryptamine; $\circ$, 5-methoxyindole; $\square$, 5-hydroxytryptamine; $\bullet$, melatonin; $\blacksquare$, 6-F melatonin.

These results provide the first evidence for melatonin binding in several gonadal and endocrine tissues of rodents and in human ovary. This finding correlates well with earlier studies which showed the presence of ^3H -melatonin in cytosols prepared from ovary, eye and pineal taken from various mammalian species^{10,11}. The tissue specificity of this binding is of particular interest since it correlates well with the previously documented suppressive effects of melatonin on gonadal tissues. The cytoplasmic location of this binding activity is unique for a nonsteroidal biogenic amine, and contrasts with the membrane binding site of compounds such as dopamine, histamine, catecholamines and acetylcholine¹². Our preliminary studies of the structural specificities of this binding activity suggest a requirement for the indole nucleus and decreased affinity for indole derivatives lacking the *N*-acetyl group. The finding that 6-fluoromelatonin competes equally with ^3H -melatonin for binding is consistent with the established ability of this analogue to suppress LH secretion by the pituitary in a manner similar to the action of melatonin⁶.

These results support the hypothesis that melatonin exerts direct regulatory actions on peripheral gonadal and endocrine tissues. In view of reported melatonin inhibition of tumour growth¹³, it will be important to examine malignant tissues for the presence of receptor activity.

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Specific suppression of antigen-reactive cells in neonatal transplantation tolerance

ANALYSES of allogeneic immunological reactivity of neonatally tolerant animals at the cellular level have produced mixed results. Some investigators have observed complete nonreactivity and others have described variable, though well defined, specific reactivity in the mixed lymphocyte culture or in the cytotoxicity test. These findings can be explained by the different degree of tolerance achieved^{1,2} and by the different intensity of the suppressor mechanisms which may be involved in neonatal transplantation tolerance^{3–5}. We report here an analysis of immunological reactivity in two experimental systems of neonatal tolerance in mice involving the disparity in either the entire *H-2* complex or the *D* region only. We find that cells from tolerant animals nonreactive in the cytotoxicity test *in vitro* can be separated into nonadherent and nylon-adherent cells according to adherence to nylon wool. The former cells, when sensitised *in vitro*, are cytotoxic, as are the

Table 1 Nonreactivity of lymphoid cells from neonatally tolerant mice in the microcytotoxicity test

Expt	Cells tested*	Survival of B10 target cells (c.p.m. per well)	% Cyto-toxicity	P†
1	Normal B10.A	1,765 ± 144		
	B10.A tolerant to B10	1,669 ± 215	5.5	NS
	B10.A immune to B10	636 ± 75	72.6	<0.001
2	Normal B10.A	1,703 ± 160		
	B10.A tolerant to B10	1,551 ± 153	8.9	NS
3	Normal B10.A	1,727 ± 186		
	B10.A tolerant to B10.A(2R)	1,486 ± 105	14.0	NS
	B10.A immune to B10.A(2R)	467 ± 45	73.0	<0.001
4	Normal B10.A	1,475 ± 168		
	B10.A tolerant to B10.A(2R)	1,555 ± 211	–5.4	NS

NS, not significant. Survival given as mean ± s.e.m.

* Lymphoid cells from normal, tolerant or immune (10 d after transplantation) B10.A mice were tested at the effector: target cell ratio of 100:1.

† Statistical significance (*P*) determined using Student's *t* test.

Table 2 *In vitro* sensitisation of cells separated in nylon wool columns according to adherence properties

Group	Responding cells*	Stimulating cells	Survival of B10 target cells (c.p.m. per well)	% Cyto-toxicity	P
A	Tolerant nonseparated	B10.A	694 ± 110		
		B10	569 ± 74	18.0	NS
B	Tolerant nonadherent	B10.A	1,377 ± 124		
		B10	643 ± 66	53.3	<0.001
C	Tolerant nylon-adherent	B10.A	869 ± 51		
		B10	1,005 ± 56	-15.9	NS
D	Normal nonseparated	B10.A	1,470 ± 188		
		B10	784 ± 58	46.7	<0.010
E	Normal nonseparated + tolerant nonseparated (1:1)	B10.A	639 ± 83		
		B10	428 ± 43	33.1	<0.050
F	Normal nonseparated + tolerant nonseparated (2:1)	B10.A	726 ± 90		
		B10	399 ± 70	45.0	<0.025

Cells tested were from B10.A mice tolerant to B10 antigens. NS, Not significant.

* After *in vitro* sensitisation cells were tested in the microcytotoxicity test at the effector: target cell ratio of 50:1.

sensitised cells from normal animals. The latter remain nonreactive even after the sensitisation and, in addition, specifically inhibit the *in vitro* sensitisation of cells from normal animals.

Mice of the inbred strains B10.A, B10.A(2R) and C57BL/10ScSn (referred to here as B10) have the same genetic background B10 and differ only by H-2 antigens. Tolerance was induced in newborn (up to 20 h after birth) B10.A mice by the intravenous injection of $12-15 \times 10^6$ living spleen cells from (B10.A × B10)F₁ or /B10.A × B10.A(2R)/F₁ hybrid mice. At 8-9 weeks of age, mice were grafted with allogeneic skin⁶. Mice neonatally treated with (B10.A × B10)F₁ cells retained approximately 50% of the B10 grafts for more than 100 d, and those injected with /B10.A × B10.A(2R)/F₁ cells accepted approximately 95% of the B10.A(2R) grafts permanently⁷. Only mice bearing grafts without any cosmetic defects for more than 100 d were used in the *in vitro* experiments. These mice did not react by significant cytotoxic activity against cells carrying the tolerated antigens (Table 1).

Lymph node and spleen cell suspensions were prepared from individual mice in RPMI 1640 medium supplemented with 5% fetal calf serum (SEVAC). Cells were washed once and approximately half were separated according to adherence to nylon wool as described by Julius *et al.*⁸. Cells were incubated in nylon wool columns for 45 min at 37 °C and the nonadherent

cells were washed through with the medium. The nylon wool columns were washed with excess medium, and the adherent cells were collected by thorough washing. Cells to be sensitised (1.5×10^6) were incubated with 0.4×10^6 irradiated (2,000 R) stimulating cells in 1 ml of RPMI medium supplemented with 10% fetal calf serum, streptomycin, penicillin and 5×10^{-5} M 2-mercaptoethanol. After 4 d of incubation at 37 °C in an atmosphere of 5% CO₂ in air, the cells were collected and their cytotoxicity measured in the microcytotoxicity assay⁹ with the modification that ³H-thymidine was used to label surviving target cells. The target cells derived from a sarcoma induced by methylcholanthrene in B10 mice possessed the same H-2 antigens as B10 cells used for sensitisation of lymphocytes and the same D region as B10.A(2R) cells. After incubating target cells together with the lymphocytes tested, the activity incorporated in the surviving target cells was counted¹⁰.

In the first experiment, transplantation tolerance was analysed in the donor B10-recipient B10.A combination with the difference in the entire H-2 complex. Cells from these tolerant mice did not develop cytotoxic activity when sensitised *in vitro* to the tolerated antigens (Table 2, group A). After separating adherent and nonadherent cells from the nonreactive cells by nylon wool passage before sensitisation, the nonadherent population showed strong cytotoxicity (Table 2, group B) and the adherent population remained nonreactive

Table 3 *In vitro* sensitisation of cells separated in nylon wool columns according to adherence properties

Group	Responding cells*	Stimulating cells	Survival of B10 target cells (c.p.m. per well)	% Cyto-toxicity	P
A	Tolerant nonseparated	B10.A	1,496 ± 153		
		B10.A(2R)	1,505 ± 137	-0.6	NS
		B10	646 ± 50	56.8	<0.001
B	Tolerant nonadherent	B10.A	2,787 ± 184		
		B10.A(2R)	2,024 ± 195	27.4	<0.025
		B10	1,385 ± 122	50.3	<0.001
C	Tolerant nylon-adherent	B10.A	4,289 ± 296		
		B10.A(2R)	4,175 ± 277	2.7	NS
		B10	2,270 ± 164	47.1	<0.001
D	Normal nonseparated	B10.A	2,346 ± 162		
		B10.A(2R)	1,169 ± 193	50.9	<0.001
		B10	1,054 ± 95	55.1	<0.001
E	Normal nonseparated + tolerant nylon-adherent (1:1)	B10.A	1,442 ± 151		
		B10.A(2R)	1,383 ± 170	4.1	NS
		B10	805 ± 64	44.2	<0.001

Cells tested were from B10.A mice tolerant to B10.A(2R) antigens. Nonadherent cells react and nylon-adherent cells do not react. Specificity of tolerance and specific suppression of sensitisation of cells from normal mice by nylon-adherent cells from tolerant mice. NS, Not significant.

* *In vitro* sensitised cells were tested in the microcytotoxicity test at the effector: target cell ratio of 50:1.

(Table 2, group C) after sensitisation to the tolerated antigens. Nonadherent cells from tolerant animals were as reactive as sensitised cells from normal animals (Table 2, group D). In addition, the sensitisation of cells from normal mice was partially inhibited by adding cells from tolerant animals (Table 2, groups E and F). The results were confirmed when the experiment was repeatedly performed.

In the second experiment, transplantation tolerance was analysed in the donor B10.A(2R)-recipient B10.A combination with the D-region difference only. Cells from tolerant mice could not be sensitised to the tolerated antigens (Table 3, group A), but they normally reacted to antigens of a third-party strain B10. After separation of two populations of cells from tolerant animals by nylon wool columns, the nonadherent cells reacted to the tolerated antigens and antigens of a third-party strain (Table 3, group B) and the nylon-adherent cells did not react (Table 3, group C) after sensitisation. Sensitised cells from normal B10.A mice exhibited the same reactivity to B10 and B10.A(2R) antigens (Table 3, group D). However, when cells from normal animals were incubated together with the nylon-adherent cells from tolerant animals, they were not cytotoxic for the antigens tolerated by the nonreactive cells, but reacted normally to antigens of a third-party strain (Table 3, group E). Also in this case, the results were consistent in replicate experiments.

Thus, our results show that cells capable of reacting to the tolerated antigens may be present in animals with long-lasting tolerance and that their activity may be specifically inhibited by suppressor cells. It seems that, at least in some cases, tolerance *in vivo* and unresponsiveness *in vitro* may exist without clonal deletion because the originally nonreactive cells can be sensitised *in vitro* after separation of the nylon-adherent cells.

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Binding of ^3H -neuroleptics and ^3H -apomorphine in schizophrenic brains

THE neuroleptic or antipsychotic drugs, introduced in 1952¹, effectively prevent relapse and rehospitalisation of schizophrenic patients. These drugs provide a strategy² for localising possible abnormalities in the brain in schizophrenia. The hypothesis that schizophrenia might be associated with abnormally sensitive dopamine synapses³⁻⁶ evolved from the suggestion that neuroleptics block dopamine receptors and thus accelerate the turnover of dopamine^{7,8}. The hypothesis received additional support from the observations that dopamine-mimetic drugs (for example *d*-amphetamine, L-dopa) elicit or exacerbate psychotic symptoms⁹⁻¹², that neuroleptics produce supersensitivity to dopamine¹³⁻¹⁵, that the neuroleptic and antipsychotic actions are enhanced by drugs which block the synthesis of catecholamines¹⁶, that

neuroleptics all have conformations that match that of dopamine¹⁷, and that dopamine is the most potent neurotransmitter to inhibit the specific binding of ^3H -haloperidol to dopamine-rich regions of the brain¹⁸⁻²⁰. Although all this evidence for the dopamine hypothesis of schizophrenia is circumstantial, we have now obtained direct evidence for some abnormalities in brain dopamine receptors in schizophrenia by measuring the specific binding of ^3H -apomorphine and ^3H -haloperidol or ^3H -spiperone to four regions of postmortem brains from schizophrenic patients.

A total of 28 neurologically normal and 20 schizophrenic postmortem brains were analysed. The 28 control subjects were aged 19-80 yr (mean 46 yr) and the 20 schizophrenia patients 22-92 yr (mean 51 yr). The interval between death and freezing of the brain was 6 to 85 h (mean 36 h) for controls, and 4.5 to 60 h (mean 29 h) for schizophrenic tissues. The causes of death in controls were car accidents, pneumonia or myocardial infarction; the causes of death in the schizophrenic patients were generally suicide (but not with drugs), myocardial

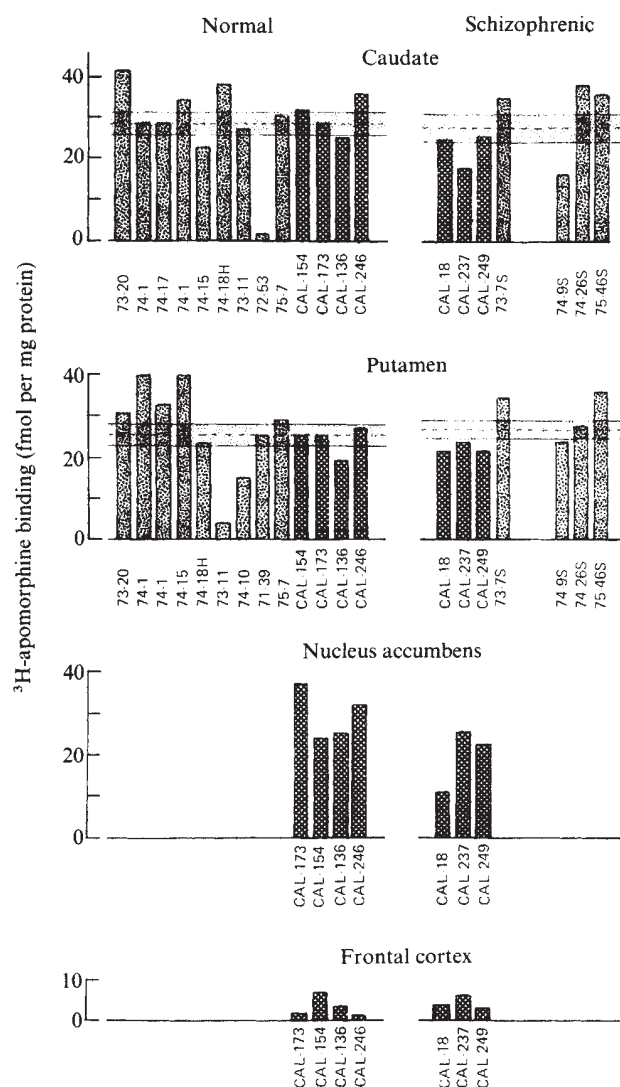


Fig. 1 Specific binding of ^3H -apomorphine was approximately the same in postmortem brain from neurologically normal and schizophrenic patients. Each column represents the result for one patient, averaging 4 to 10 separate assays (in sextuplicate) per tissue. The medium grey columns represent tissues from the Clarke Institute of Psychiatry and the dark columns indicate those from the Wadsworth Hospital. The horizontal lines, interrupted and solid, indicate the mean and s.e.m. respectively, for each group.

infarction or pneumonia. The concentrations of dopamine in the striatum of four schizophrenic subjects (73-7S, 74-9S, 74-26S, 75-46S) ranged from 3.7 to 8.4 μg per g in the caudate and 4.9 to 6.3 in the putamen, all values being within normal limits. Additional data are given in Table 1.

Although there was no difference between normal and schizophrenic brains for ^3H -apomorphine (Fig. 1), there was a significantly higher amount of specific ^3H -haloperidol binding in the three main dopamine-rich regions of the schizophrenic brains (Fig. 2). The average increase was 74% in the caudate

($P < 0.001$), 51% in the putamen ($P < 0.001$), and 71% in the nucleus accumbens. Most of the patients had been receiving neuroleptic drugs for some time. Two patients (S-14, S-15) had apparently not received any neuroleptics, and three had only received neuroleptics in the last 24–48 h before death. Although these few untreated schizophrenic tissues also revealed increased binding of ^3H -haloperidol, they are too few in number to consider as a separate group; moreover, it is not certain that such patients did not receive neuroleptics some weeks before death.

Table 1 Postmortem analysis of caudate nuclei

Brain no.	Age of patient (yr)	Interval between death and freezing (h)	Duration of frozen storage	Ratio of specific to nonspecific binding for 1.7 nM ^3H -haloperidol ($\times 100\%$)	Specific binding of ^3H -spiperone at 1 nM (fmol per mg)	Ratio of specific to nonspecific binding for ^3H -spiperone ($\times 100\%$)
Normals*						
C-93 (F)	38	48?	20 months(mo.)	10.7 $\pm$ 1.6	98.3 $\pm$ 4.9	17.4 $\pm$ 0.9
C-101 (M)	20	85	19 mo.	14.6 $\pm$ 2.2	111.6 $\pm$ 13.7	16.9 $\pm$ 1.5
C-105 (F)	—	48?	18 mo.	13.2 $\pm$ 1.9		
C-110 (M)	72	81	17 mo.	6.7 $\pm$ 0.9		
C-113 (M)	66	25	17 mo.	14.6		
C-114 (M)	55	32	17 mo.	9.6 $\pm$ 1.1		
C-104 (M)	19	48?	18 mo.	16.5 $\pm$ 3.0	48.2 $\pm$ 12.6	13.4 $\pm$ 1.0
C-135 (M)	77	48?	9 mo.	13.1 $\pm$ 1.4	141.7 $\pm$ 15.7	18.7 $\pm$ 2.5
C-140 (M)	—	48?	7 mo.	8.5 $\pm$ 2.5		
C-141 (M)	80	48?	6 mo.	10.3 $\pm$ 1.1	95.0 $\pm$ 8.1	15.2 $\pm$ 1.3
73-11 (M)	67	6	4 yr	13.1 $\pm$ 0.8		
75-7 (M)	62	14	2 yr	12.3 $\pm$ 2.5		
74-1 (M)	25	24	3 yr	15.5 $\pm$ 0.8		
73-16 (M)	66	8	4 yr	13.6 $\pm$ 2.4		
73-23 (M)	20	20	4 yr	14.5 $\pm$ 3.6		
73-20 (M)	55	7	4 yr	11.5 $\pm$ 1.7		
73-13 (M)	19	17	4 yr	13.2 $\pm$ 1.6		
Cal-154 (—)	—	48?	14 mo.	8.3 $\pm$ 2.5		
Cal-173 (M)	26	6	33 mo.	11.6 $\pm$ 2.3		
Cal-136 (F)	40	48	47 mo.	13.5 $\pm$ 1.4		
Cal-246 (F)	25	48	5 mo.	8.9 $\pm$ 2.0		
Average	46	36 $\pm$ 5	26 $\pm$ 3.5 mo.	12.1 $\pm$ 0.6	98.9 $\pm$ 15.0	16.3 $\pm$ 0.9
Schizophrenic						
S-6 (M)	22	60	19 mo.	18.2 $\pm$ 2.4		
S-7 (F)	50	78	17 mo.	21.1 $\pm$ 3.1	259.9 $\pm$ 40.0	29.1 $\pm$ 4.8
S-9 (F)	85	34	15 mo.	12.8 $\pm$ 1.8	202.2 $\pm$ 20.8	23.0 $\pm$ 2.6
S-13 (M)	67	48?	11 mo.	16.9 $\pm$ 2.0	199.5 $\pm$ 16.5	25.4 $\pm$ 2.2
S-18 (F)	80	37	9 mo.	11.6 $\pm$ 1.7	147.3 $\pm$ 12.2	22.3 $\pm$ 1.9
S-23 (M)	56	49	8 mo.	18.9 $\pm$ 2.1	118.7 $\pm$ 8.9	16.8 $\pm$ 1.3
S-24 (M)	64	14	8 mo.	20.6 $\pm$ 2.2	112.6 $\pm$ 15.8	17.9 $\pm$ 2.8
S-26 (M)	51	24	6 mo.	17.5 $\pm$ 1.4	175.9 $\pm$ 15.0	31.3 $\pm$ 2.1
73-17s (M)	17	21	4 yr	18.2 $\pm$ 3.6		
73-7s (F)	24	16	4 yr	21.9 $\pm$ 2.0		
Cal-18 (F)	74	8	11 yr	20.0 $\pm$ 4.5		
Cal-237 (F)	48	12	6 mo.	24.6 $\pm$ 1.9		
Cal-249 (M)	22	17	5 mo.	23.4 $\pm$ 3.4		
S-12 (M)	44	17	11 mo.	20.1 $\pm$ 1.0	116.9 $\pm$ 13.4	16.9 $\pm$ 1.9
S-14 (F)	70	48?	11 mo.	18.4 $\pm$ 1.4	94.6 $\pm$ 7.5	17.4 $\pm$ 1.6
75-46s (M)	75	7	2 yr	11.6 $\pm$ 1.2		
74-26s (M)	24	5	3 yr	29.4 $\pm$ 1.8		
74-9s (F)	45	25	3 yr	14.8 $\pm$ 0.5		
Average	51	29 $\pm$ 5	25 $\pm$ 7.1 mo.	18.9 $\pm$ 1.1	158.6 $\pm$ 18.0	22.2 $\pm$ 1.8

Frozen samples of postmortem human brain tissue were thawed and suspended in buffer (1 ml per 20 or 50 mg tissue). The buffer contained 15 mM Tris-HCl, pH 7.4, 5 mM Na_2EDTA , 1.1 mM ascorbic acid and 12.5 μM nialamide. After homogenisation in a glass-Teflon homogeniser, the 1 ml sample was pre-incubated for 1 h at 37 °C and then frozen at –20 °C. For the radio-receptor assay the homogenate was thawed, resuspended, and centrifuged at 39,000g for 15 min at 4 °C. The pellet was resuspended in 2 ml buffer and homogenised with a Polytron PT-10 at a setting of 5.5 (full-scale = 10) for 20 s. Aliquots (0.2 ml) of this final homogenate were added to 12 $\times$ 75 mm tubes containing 0.2 ml of ^3H -apomorphine²¹ (14.1 Ci mmol^{–1}; final concentration 3.0–3.3 nM) or 0.2 ml of ^3H -haloperidol (7.8 Ci mmol^{–1}; prepared in May 1974, by IRE Belgique with specific activity of 9.6 Ci mmol^{–1}; 1.7 nM final concentration), and 0.2 ml of buffer with or without (+)butaclamol (the final concentration of which was 100 nM for the ^3H -haloperidol assay, and 1 μM for the ^3H -apomorphine assay). There was only sufficient tissue (caudate) remaining for a few measurements of specific ^3H -spiperone binding. This was done using a final concentration of 1 nM ^3H -spiperone (36 Ci mmol^{–1}; NEN) and 1 μM (+)butaclamol to define specific binding²²; the filters were washed with 15 ml buffer. Specific binding for each radioligand was defined as the total binding minus that which occurred in the presence of (+)butaclamol. Putamen and caudate assays were repeated four to ten times, each assay in sextuplicate. The criteria used for diagnosis of schizophrenia in this series have been outlined in ref. 23.

* M, male; F, female.

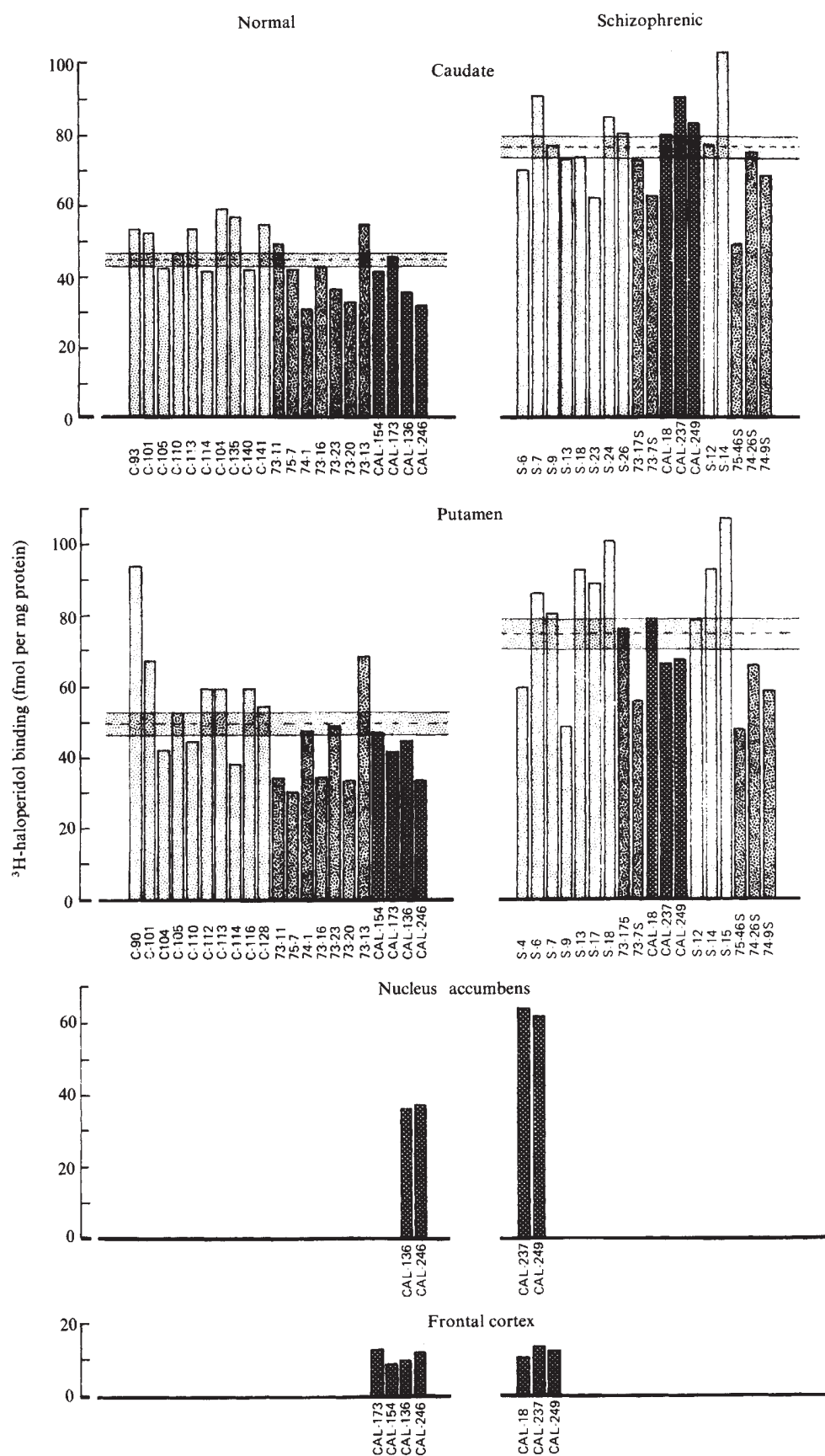


Fig. 2 Evidence for an increase in specific ^3H -haloperidol binding in three dopamine-rich regions of postmortem brains from schizophrenic patients. Each column indicates the average of 4 to 10 separate assays (in sextuplicate) for each patient's brain region. The light-grey columns represent tissues from Addenbrooke's Hospital; the medium-grey those from the Clarke Institute of Psychiatry; and the dark columns, from Wadsworth Hospital. The horizontal lines, interrupted and solid, indicate the mean and s.e.m., respectively, for each group. The specific binding of ^3H -haloperidol in the schizophrenic caudate is higher by 74% ($P < 0.001$). Specific binding in the putamen was higher by 51% ($P < 0.001$).

There was only sufficient tissue remaining to measure specific ^3H -spiperone binding in 5 normal putamens, 3 normal caudates, 9 schizophrenic putamens and 9 schizophrenic caudates. Data for the caudates are listed in Table 1. The specific binding of ^3H -spiperone was increased by 59% in the 9 schizophrenic caudates (Table 1) and by 75% in the 9 schizophrenic putamens (data not shown).

The average percent specific binding for ^3H -haloperidol was 12.1% for normal and 18.9% for the schizophrenic tissues (based on data after the background adsorption of ^3H -haloperidol to the GF/B filters was subtracted). This specific binding is about the same as that found for calf caudate tissues when the ^3H -haloperidol radioreceptor assay was developed previously in this laboratory^{18,19}. Although the specific binding for ^3H -spiperone is higher than that for ^3H -haloperidol, the difference between control and schizophrenic tissues is similar. A typical numerical example for one control brain is as follows. Eight separate determinations were made in sextuplicate on eight different days. The total and nonspecific amounts of ^3H -haloperidol binding, after subtracting that adsorbed to filters, were, respectively, 590 and 550; 629 and 541; 412 and 358; 392 and 345; 488 and 424; 419 and 391; 540 and 475; 456 and 388 fmol per mg protein. The mean $\pm$ s.e.m. were 490 ± 30.9 and 434.0 ± 28.1 , whereafter the specific binding was 56.7 ± 6.6 fmol per mg; ratio of specific to nonspecific binding was 13.1% (brain C-135 Table 1).

As there was insufficient tissue to carry out a complete range of ^3H -haloperidol test concentrations, it is not known whether the increased binding represents an increased number of neuroleptic receptors or an increased affinity of the neuroleptic receptors for ^3H -haloperidol. There was apparently no qualitative difference in the neuroleptic sites between the control and the schizophrenic tissues. For example, the dissociation constants (in selected normal and schizophrenic putamens) were 2 nM for ^3H -haloperidol and 0.3 nM for ^3H -spiperone in both.

Although these results are apparently compatible with the dopamine hypothesis of schizophrenia in general, much, if not all, of the increased ^3H -neuroleptic binding may have resulted from the long-term administration of neuroleptics^{24,25}. Long-term neuroleptic treatment, however, does elevate ^3H -apomorphine by 80% (ref. 26), but the binding of ^3H -apomorphine was normal in the schizophrenic tissues.

Since most evidence indicates that neuroleptics and apomorphine both attach to dopamine receptors, it is at first surprising that the binding of ^3H -apomorphine does not change (Fig. 1) as does that for ^3H -haloperidol (Fig. 2). Recent evidence indicates, however, that ^3H -apomorphine has a predilection for presynaptic dopamine receptors²⁷; thus ^3H -apomorphine and ^3H -haloperidol may be measuring preferentially pre- and postsynaptic dopamine receptors, respectively. This is consistent with the recent observation that low doses of apomorphine do not exacerbate but may alleviate some psychotic symptoms¹¹.

These results cannot prove that dopamine receptors are intrinsically increased in schizophrenic patients in the absence of the neuroleptics, but they do at least provide considerable support for the notion that neuroleptic-induced tardive dyskinesia may stem from a drug-induced increase in dopaminergic sensitivity¹³.

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Ventromedial hypothalamus modulates fat mobilisation during fasting

THE weight of adipose tissue is regulated by both hormonal and neural mechanisms^{1,2}. The sympathetic nerve fibres which innervate adipose tissue³ enhance lipolysis when stimulated^{4,5}. Denervation, on the other hand, slows the rate of fatty acid mobilisation during fasting^{6,7} and complete exclusion of the sympathetic nervous system is followed by an increase in weight⁸. The possibility that the medial or lateral hypothalamus (LH) is involved in modulating the sympathetic innervation to fat tissue has been suggested by the discovery that bilateral injury to the ventromedial hypothalamus (VMH) attenuated fatty acid mobilisation after stress⁹. If either of these hypothalamic areas were involved in regulating lipolysis, then unilateral denervation of the abdominal adipose tissue might produce effects qualitatively similar to unilateral hypothalamic injury. We have tested this hypothesis and report here that unilateral abdominal sympathectomy or an ipsilateral ventromedial hypothalamic lesion impairs lipolysis during fasting but that a lateral hypothalamic lesion is without effect.

The adrenal medulla was removed on both sides from 80 male rats to remove the primary source of circulating adrenaline, and a further 16 rats had sham operations. Five to six weeks later, with the rats under ether anaesthesia, a lumbar sympathectomy was performed on the left side of 16 of the original 80 rats, with the aid of a binocular dissecting microscope. All visible neural connections along the left side of the aorta and to the left perirenal and retroperitoneal adipose tissue were severed⁶. The other 64 rats received sham operations which consisted of opening and closing the abdomen without disturbing the

Table 1 Effect of fasting for 96 h on weight of epididymal and retroperitoneal fat pad

Group	n	Weight of fat pads (g)					
		Epididymal		P*	Retroperitoneal		P*
		Right	Left		Right	Left	
Control	8	4.86 ± 0.70	4.90 ± 0.75	NS	6.15 ± 0.98	6.18 ± 0.91	NS
Sympathectomy	8	4.45 ± 0.43	4.60 ± 0.43	NS	4.90 ± 0.31	5.78 ± 0.46	<0.01
Unilateral VMH	8	4.68 ± 0.39	4.83 ± 0.46	NS	5.36 ± 0.51	5.79 ± 0.59	<0.01
Bilateral VMH	8	5.36 ± 0.59	5.36 ± 0.60	NS	7.16 ± 0.82	7.14 ± 0.87	NS
Unilateral LH	8	4.59 ± 0.60	4.63 ± 0.57	NS	5.88 ± 0.93	6.02 ± 0.97	NS
Bilateral LH	8	4.38 ± 0.61	4.25 ± 0.55	NS	5.33 ± 0.92	5.41 ± 0.95	NS

Figures represent mean ± s.e.m., NS, not significant.

*Paired comparison between left and right fat pads. Statistical significance at $P \leq 0.05$.

sympathetic fibres. Two days later electrolytic lesions were made in the ventromedial ($n = 16$, bilateral; $n = 16$, unilateral) or lateral ($n = 16$, bilateral; $n = 16$ unilateral) hypothalamus using coordinates and techniques described in detail elsewhere^{9,10}. In our hands VMH lesions are followed by obesity in 75–90% of animals and LH lesions, when these coordinates and current flow are used, produce total aphagia. Half the rats ($n = 48$) were fasted for 48 h and the other half ($n = 48$) for 96 h from the completion of surgery. At decapitation, blood was collected in chilled tubes. After examining the surgical field under $\times 20$ magnification for residual sympathetic nerves, we removed the left and right kidneys and the perirenal and epididymal fat pads, and weighed them individually. Glucose was measured by the glucose oxidase method on a Beckman glucose analyser, and free fatty acids by titration¹¹.

Kidneys were weighed as a check on the surgical procedure: those from left and right had the same weight in rats of all groups. Paired *t* tests showed that denervation significantly affected the weight of the retroperitoneal fat pads (Fig. 1, Table 1) but not the epididymal fat pads. In rats which had undergone unilateral abdominal sympathectomy, fasting was followed by a greater loss of weight on the innervated than on the denervated

side of the retroperitoneal fat pads ($P < 0.01$ at 48 h; $P < 0.01$ at 96 h), (Fig. 1, Table 1) but there was no difference in the weights of the epididymal fat pads (Table 1). In the animals with unilateral VMH lesions, the ipsilateral fat pad was also heavier than the contralateral fat pad ($P < 0.05$ at 48 h; $P < 0.01$ at 96 h) (Table 1, Fig. 1), but in animals with bilateral VMH lesions, there was no such difference. Unilateral lesions in the LH had no significant effect on the relative weights of fat pads, but in rats with bilateral LH lesions there was a statistically significant difference after 48 h ($P < 0.05$): that was opposite in direction from the effect of a unilateral VMH lesion or sympathectomy. After 96 h of fasting, no difference was detected in rats with LH lesions. Mean serum glucose ranged between 114 mg dl^{-1} and 118 mg dl^{-1} in all groups after fasting for 48 h; after 96 h it was between 91 mg dl^{-1} and 110 mg dl^{-1} . Mean free fatty acids were not significantly different after 48 h of fasting ($570\text{--}634 \mu\text{Eq l}^{-1}$). With continued fasting, all groups showed a significant increase ($P < 0.05$), except the rats with bilateral VMH lesions.

An ipsilateral VMH lesion mimicked the effect of unilateral sympathetic denervation on the weight of the retroperitoneal fat pad during fasting. This suggests that one component of the control of the weight of the adipose tissue during starvation is mediated through the VMH and its connections to the sympathetic nervous system¹². The response of the epididymal fat pad was at variance with that of the retroperitoneal pad. These two pads also respond differently to feeding diets containing a high content of fat¹³, and in genetic obesity¹⁴ where changes are greatest in the retroperitoneal fat pad with much smaller or no responses in the epididymal fat tissue. This may reflect differences in neural innervation of these two fat deposits or in the characteristics of the fat cells themselves.

Although the sympathetic nervous system has long been implicated in the control of lipolysis^{4–7}, our results constitute the first physiological evidence for a link with the VMH. Additional support for the hypothesis that this region of the hypothalamus modulates the sympathetic nervous system comes from the observation that in rats with bilateral VMH lesions the mobilisation of fatty acids is impaired after fasting or acute stress⁹. One implication of our study is that obesity which follows bilateral VMH lesions may be in part due to 'reduced' sympathetic activity and the resulting reduction in lipolysis. The compromised mobilisation of free fatty acids during fasting in rats with bilateral VMH lesions might serve as a signal to eat more calories to provide for the perceived 'metabolic deficit'.

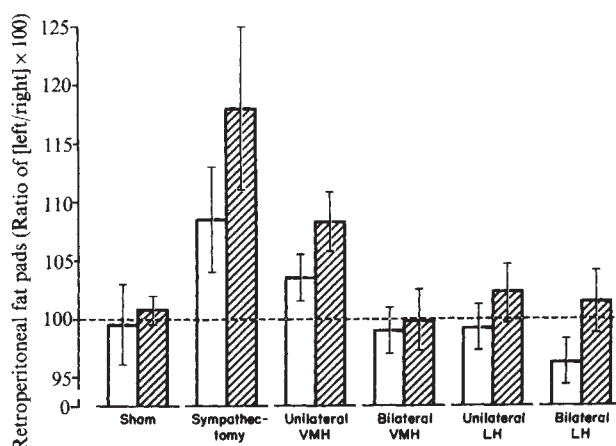
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Fig. 1 Effects of 48 h (open columns) and 96 h (hatched columns) of fasting on the weight of retroperitoneal fat pads of rats. The weight of the left to the right pad is compared for each of the experimental procedures and expressed as a percentage of the unoperated side. Each bar represents the mean ± s.e.m. for eight rats. Unilateral sympathectomy significantly slowed the loss of weight of the denervated (left) fat pad during fasting ($P < 0.01$). A unilateral (ipsilateral) VMH lesion in rats with intact sympathetic nerves produced a qualitatively similar effect to unilateral sympathectomy ($P < 0.05$). Rats with bilateral VMH lesions showed no difference. The difference in fat pad weight after 48 h of fasting in rats with unilateral LH lesions was not present in rats fasted 96 h.



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Synaptic channel gating differences at snake twitch and slow neuromuscular junctions

TWO readily distinguishable skeletal muscle fibre types are found in the muscles of lower vertebrates, including amphibians, birds, fishes and reptiles¹: twitch and slow fibres. In response to membrane depolarisation the twitch fibre contracts briefly, whereas the slow fibre can develop and maintain tension for long periods. The slow fibres each have several endplates which differ anatomically from the twitch fibre's single endplate^{1,2} and may have a direct role in the maintained contractile behaviour. We report here studies of the synaptic conductance change mediated by acetylcholine (ACh, the natural chemical agonist) in these fibres and document a difference in the voltage dependence of endplate channel lifetime which correlates with muscle type. This difference may be mediated by an altered expression of the gating mechanism of the synaptic channel.

Experiments were carried out on twitch and slow fibres in the costocutaneous muscle of garter snakes (sp. *Thamnophis*). Whole muscles were removed with a portion of the rib and scale on which they inserted and mounted in a small (4 ml) glass-bottomed plexiglass chamber filled with snake physiological solution, composition (mM): NaCl 159; KCl 2.15; CaCl₂ 1.0; MgCl₂ 4.2; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) 1.0, at pH 7.2 and temperature 12–16 °C. In some experiments the bath contained tetrodotoxin 100 nM to block sodium channels. With the muscle spread laterally neuromuscular junctions on single fibres were visualised with modulation contrast optics³ and voltage-clamped with two microelectrodes. In medium sized snakes these muscles were composed of 50–100 cells, approximately 40% of which were slow fibres. Twitch and slow muscle fibres may be distinguished by anatomical, mechanical and electrical criteria¹. In practice we identified slow fibres by their innervation and confirmed that identification with observations of contractile behaviour. Slow and twitch muscle fibres could also be distinguished with fair success on size alone. In these snakes slow fibres were typically 10–20 µm in diameter whereas twitch fibres ranged between 30–50 µm. The neuromuscular junctions of both fibre types were compact relative to their space constants (1–4 mm)⁷, allowing the voltage clamp to provide effective voltage control over the entire active synaptic region. Spontaneous miniature endplate currents (m.e.p.c.s) and drug-induced endplate current (e.p.c.) during the iontophoretic application of ACh were digitally recorded and analysed for information on mean channel lifetime. Standard methods of noise analysis were used^{4,5,6}.

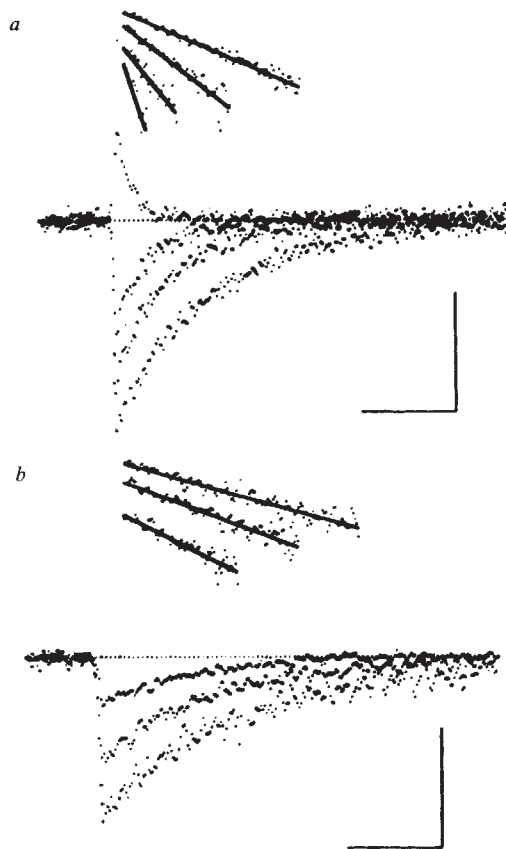
Miniature e.p.c.s from several different neuromuscular junctions could be recorded at a single site in the multiply innervated slow muscle fibres. M.e.p.c.s with the most rapidly increasing phase were selected for analysis; these were deemed to have occurred at the neuromuscular junction next to the recording electrodes. Slow rising m.e.p.c.s were attributed to

distant junctions, the slowing due to the filter-like properties of the muscle between the m.e.p.c. site and recording electrodes. Whereas slowly increasing m.e.p.c.s were common in slow muscle fibres, they were rarely recorded in twitch fibres.

It was initially observed that slow fibre m.e.p.c.s decayed more slowly and with less voltage sensitivity than those from twitch fibres in the same muscle (Fig. 1). From the time courses, it seems that decay is reduced as the membrane voltage is made more positive in both fibre types. However, the voltage sensitivity of decay is greater in the twitch fibre so that a more extreme change is seen with voltage than in the slow fibre. Above the m.e.p.c. time courses in Fig. 1 the currents are plotted semilogarithmically. The different voltage sensitivity of m.e.p.c. decay is seen here as an unequal change in the slope of these plots with voltage.

Note that both the twitch and slow fiber m.e.p.c.s decay exponentially with time. Averaged m.e.p.c. decays could be fitted with the expression $I(t) = I(0) \exp(-t/\tau)$ where $I(0)$ is the peak current and τ is the decay time constant given by the semilogarithmic slope. Thus the different time courses of twitch

Fig. 1 Time course of m.e.p.c.s in twitch and slow muscle fibres. Averaged m.e.p.c.s of 16–20 individuals each from representative twitch (a) and slow (b) muscle fibres reveal gross similarities and subtle differences in the time course of their decay and voltage sensitivity. The m.e.p.c. records at several voltages are displayed with superimposed baselines; above each set of records a portion of the decay is plotted semilogarithmically and fitted with a least squares regression line to evaluate the decay time constant. The linearity of this semilogarithmic plot indicates that m.e.p.c.s in both fibres decay exponentially with time. The differing voltage dependences of τ at twitch and slow fibre junctions is evident in the amount of change in the semilogarithmic slopes with voltage. From bottom to top, twitch fibre data: -118, -88, -48, +25 mV; slow fibre data: -98, -57, -23 mV (14 °C). Calibration marks: 2 nA, 5 ms.



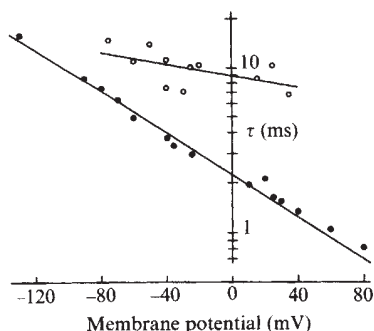


Fig. 2 Voltage dependence of decay time constant, τ for both twitch and slow fibre m.e.p.c.s depends exponentially on voltage. This dependence was greater and characteristically less variable in twitch fibres (●) than in slow fibres (○); data here were chosen to illustrate the mean voltage sensitivities found in the two fibre types. Equation (1) has been fitted to both sets of data: slow $\tau_0 = 8.66$ ms, $A = 0.004$ mV $^{-1}$; twitch $\tau_0 = 2.33$ ms, $A = 0.014$ mV $^{-1}$ (14°C).

and slow fibre m.e.p.c.s do not arise from the appearance of several kinetic steps such as occurs when local anaesthetics alter the e.p.c.^{5,8,9}, although they could arise if the rate limiting step in the decays were different. We tested this possibility with the following results.

The dependence of τ on voltage is illustrated more clearly in Fig. 2. With twitch fibres the voltage dependence of τ was accurately described by the exponential function

$$\tau(V) = \tau_0 e^{-AV} \quad (1)$$

in a way identical to Magleby and Stevens' description¹⁰ of e.p.c. decay in frog twitch muscle fibres. Although in slow fibres τ showed greater scatter than in twitch fibres (possibly related to difficulties of recording from slow fibres for long periods), within this scatter the voltage dependence of slow fibre decays was also reasonably consistent with equation (1). The grand mean values and ranges of the decay amplitude coefficient τ_0 (ms) and the voltage coefficient A (mV $^{-1}$) are given in Table 1. Because there was overlap in the coefficients between twitch and slow fibres, seven experiments (20 cells) were selected in which both fibre types were studied in the same muscle. We always observed that within a muscle the slow fibre m.e.p.c.s decayed more slowly and with less voltage sensitivity than the twitch fibre m.e.p.c.s. For these seven muscles the average coefficient ratio was $\tau_0(\text{slow})/\tau_0(\text{twitch}) = 2.58$ and $A(\text{slow})/A(\text{twitch}) = 0.53$, quite similar to the ratio of the grand means.

It has been well established that the m.e.p.c. decay time constant is a measure of the mean open channel duration at frog twitch fibre neuromuscular junctions⁴. This same relation may hold in both twitch and slow fibres of the snake. Mean open channel duration ($\bar{\tau}$, channel lifetime) was estimated by analysing e.p.c. fluctuations induced by iontophoretically applied ACh. In all twitch and most slow fibre junctions at negative voltages the spectral density of the fluctuations could be adequately fitted with a Lorentzian function⁴. Such a function

is characterised by its zero slope at low frequencies, its f^{-2} slope at high frequencies and its corner frequency f_0 . The corner frequency is simply related to $\bar{\tau}$ by $\bar{\tau} = 1/2\pi f_0$. The pooled data from 19 twitch fibres and 26 slow fibres showed no consistent difference between $\bar{\tau}$ estimated from m.e.p.c. decay and $\bar{\tau}$ estimated by fitting the fluctuations spectrum. A plot of τ against $\bar{\tau}$ in Fig. 3 illustrates this. Although about half the slow fibre spectra were well described by a single Lorentzian component, the rest yielded spectra which contained larger than predicted power densities at high frequencies. These more complex spectral shapes were consistent with the presence of two components, the faster being present to varying degrees. Its origin is unclear and deserves further study.

Despite the extended time course and different voltage sensitivity of m.e.p.c.s in slow fibres compared with those in twitch fibres, the decay of both provides an estimate of the apparent lifetime of synaptic channels. That is, the identity between mean channel lifetime obtained from e.p.c. fluctuations and m.e.p.c. decay time shows that the same phenomenon underlies both responses in some fibres. The extended time course of m.e.p.c.s in these fibres does not derive from a process such as the loss of cleft ACh through hydrolysis or diffusion. The same process in both fibre types determines m.e.p.c. decay, but between twitch and slow fibres the molecular mechanism of this process seems to be slightly different as evidenced by the voltage dependences.

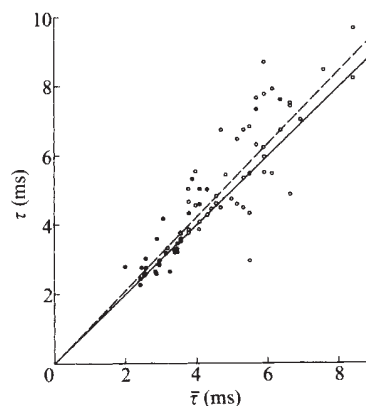


Fig. 3 Relationship between mean channel lifetime and m.e.p.c. decay. Equivalence of τ and $\bar{\tau}$ was tested by comparing estimates of each obtained at the same neuromuscular junction in identical conditions. Data are from 18 twitch and 26 slow fibre junctions, many at more than one voltage, in which both m.e.p.c.s and noise were recorded at the same voltage and temperature (15 preparations, 12–14°C, voltage range -125 to -30 mV). ○, Slow fibre data; ●, twitch fibre data. A Model II least squares regression line (dashed) constrained to pass through the origin has a slope of 1.057; the identity of τ and $\bar{\tau}$ gives a predicted slope of 1.0 (solid line).

Differences between the apparent lifetimes of channels in twitch and slow muscle fibre endplates have been observed. In addition we have looked at other parameters, including single channel conductance, which could constitute a characterisation of the endplate conductance mechanism, and have found these to be essentially identical. The subtle difference in apparent lifetimes may not be insignificant. First, it is consistent with the functional requirements of these cells. Slow muscle fibres rely on their repeated synaptic contacts to initiate and in part maintain membrane depolarisation and generate tension. If evoked conductance were too short at the depolarised voltages, sustained tension could be difficult or impossible; but apparent lifetime is not strongly altered by voltage here. In contrast, twitch fibres need to contract briefly. If the postsynaptic channels remained open too long the muscle could twitch repetitively; here depolarisation of the twitch fibre during contraction markedly accelerates the closing of its synaptic channels.

Table 1 Observed values of the coefficients in equation (1) describing twitch and slow fibre m.e.p.c. decays

	Mean $\pm$ s.e.	Range
23 twitch fibres		
τ_0 (ms)	2.42 ± 0.27	5.86–0.94
A (mV $^{-1}$)	0.013 ± 0.001	0.017–0.009
15 slow fibres		
τ_0 (ms)	4.71 ± 0.52	8.67–2.03
A (mV $^{-1}$)	0.006 ± 0.001	0.013–0.001

Second, the voltage sensitivity differences may indicate that the channel gating proteins of the two fibre types are not identical or that the environments of the gates differ. For example, if the source of lifetime voltage dependence is a conformational change of the channel protein between open and closed states¹⁰, a difference in the distributed charge on the gating portion of the protein or an alteration in the local electric field detected by the fixed gate charge could account for the results. Alternatively, changes in the ACh binding site¹¹ might produce the differences between twitch and slow fibre responses if binding, not conformational change, were rate limiting.

As the development of twitch and slow characteristics in muscle fibres seems related to a trophic effect¹² which the nerves exert on muscle, synaptic channel gating differences may be another expression of trophic influence.

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Is GABA release modulated by presynaptic receptors?

THERE is considerable evidence, in both the peripheral and central nervous systems, that presynaptic α -adrenoreceptors modulate noradrenaline release induced by both electrical and potassium ion stimulation; α -agonists inhibit and α -antagonists facilitate this release^{1–5}. There is also evidence that similar control mechanisms occur in dopaminergic^{4,6–9} and cholinergic¹⁰ neurones. Here we report that the release of γ -aminobutyric acid (GABA) from *in vitro* rat frontal cortex, caused by elevated potassium ion concentrations, is reduced by the GABA agonists muscimol and 3-aminopropane sulphonic acid, and by GABA itself, and also that the effect of muscimol is antagonised by the GABA antagonists bicuculline and picrotoxin. This *in vitro* study demonstrates that the release of GABA is subject to a negative feedback control which may be important *in vivo*, and further, that this mechanism can be modified by drugs.

The method used was essentially that of Raiteri *et al.*¹¹. Male Wistar rats were killed by cervical dislocation, and the frontal cortex was rapidly removed and chopped into 'prisms' $0.1 \times 0.1 \times \sim 2$ mm. The tissue was suspended at 10 mg wet weight per ml in incubation medium of the following composition: NaCl, 133 mM; KCl, 5 mM; CaCl_2 , 2.7 mM; MgSO_4 , 1.2 mM; Tris, 10 mM; glucose, 10 mM and aminooxyacetic acid, 0.1 mM (to prevent GABA catabolism); the pH of the medium was adjusted to 7.4 with HCl. Four 1-ml aliquots of the suspension were then added to 9 ml of medium and preincubated at 37 °C for 15 min in 25-ml conical flasks. Tritium-labelled GABA ([2,3-³H(N)]GABA, specific activity 34.5 Ci mmol⁻¹, NEN) was then added to each of the flasks to a final concentration of 10^{-8} M and the incubation continued for a further 10 min. The tissue suspensions were then poured into four parallel superfusion chambers containing at their base Millipore filters (pore size

Table 1 Drug effects on the potassium-stimulated release of GABA

Drug present	Induced release (% increase over control)	Mean drug effect on induced release
15 mM K ⁺ alone (5)	138.8 ± 12.5	
15 mM K ⁺ + 10^{-7} M muscimol (5)	112.2 ± 8.5*	– 19.0%
15 mM K ⁺ + 10^{-7} M muscimol + 10^{-6} M picrotoxin (5)	153.6 ± 13.7*†	+ 10.9%
15 mM K ⁺ alone (5)	118.5 ± 5.2	
15 mM K ⁺ + 10^{-7} M muscimol (5)	89.1 ± 5.1*	– 24.2%
15 mM K ⁺ + 10^{-7} M muscimol + 10^{-6} M bicuculline (5)	137.8 ± 7.6*‡	+ 16.1%
15 mM K ⁺ alone (4)	168.3 ± 9.3	
15 mM K ⁺ + 10^{-6} M 3-amino propane sulphonic acid (APS) (4)	120.1 ± 14.4*	– 29.7%
15 mM K ⁺ alone (4)	166.2 ± 6.7	
15 mM K ⁺ + 10^{-6} M fluspirilene + 3×10^{-7} M GABA (4)	102.2 ± 7.4§	– 37.9%

The experiments were carried out as described in the text and the value in parentheses in the first column is the number of times that the experimental condition was replicated. The second column refers to the % increase in the radioactivity released in each condition compared to control unstimulated release; in each case the mean $\pm$ s.e.m. is given and statistical significance was calculated using the matched pair Student *t* test. The third column represents the average drug-induced change compared to the release caused by potassium alone.

* $P < 0.01$, significant difference from 15 mM K⁺ alone.

† $P < 0.01$, significant difference from 15 mM K⁺ + agonist.

‡ $P < 0.001$, significant difference from 15 mM + agonist.

§ $P < 0.02$, significant difference from 15 mM K⁺ alone.

0.65 μm) supported on sintered glass discs, and thermostatically maintained at 37 °C. The excess medium was drawn off under moderate vacuum and the tissue was washed with a further 10 ml of the medium at 37 °C. A further 8 ml of medium (with the addition of fluspirilene, a GABA uptake inhibitor, in the case of experiments with GABA) was then added to each of the chambers and this was drawn through the tissue at a rate of 0.5 ml min⁻¹ using a peristaltic pump.

After 10–12 min, a steady rate of release of GABA was reached and the collection of 1-min fractions was begun. When the medium in the chambers was nearly used up, a further 10 ml of medium was introduced; this medium was either normal medium or contained 15 mM KCl (balanced by removing sodium chloride), with or without addition of the appropriate drugs. The collection of 1-min fractions was continued for a further 12 min. The remaining medium was then drawn off under vacuum and the filters with the tissue were solubilised in PCS (the sodium salt of enamine of 2,4-pentanedione and cycloserine, Amersham-Searle) overnight.

The radioactivity was determined by liquid scintillation counting with external standardisation using 30% Triton in xylene containing 0.49% 2,5-diphenyloxazole and 0.01% 1,4 di-(2-methyl styryl) benzene. Radioactivity in each fraction was calculated as a percentage of the radioactivity in the tissue at the start of the experiment. A measure of the initial phase of K⁺-induced release was taken as the area under each of the curves over a 5-min period starting from the point at which the curve reached 50% of its maximal value and expressed as a percentage increase over the corresponding area under the basal release curve. This standard procedure was used to eliminate possible anomalies which may have been caused by depletion of

^3H -GABA from the tissue stores after prolonged stimulation, although in the experiments reported the tissue content of ^3H -GABA at the end of the experiment was never less than 85% of that initially present. The data obtained were analysed using a matched pair t test and the results are shown in Table 1.

In this preparation using small tissue prisms, some part of the total GABA efflux observed may possibly have originated from glial cells. However, in cortical tissue there is good evidence that the uptake into glia is considerably less than that into neurones¹² and also indirect evidence that the K^+ -stimulated release of GABA from cortical slices is predominantly from the neuronal compartment¹³. Several workers have shown a considerable Ca^{2+} dependence of K^+ -induced ^3H -GABA release from slices or synaptosomes^{13,14}, and in the experiments reported here, in which Mg^{2+} was substituted for Ca^{2+} in the medium, a high proportion of the K^+ -induced release ($80.1 \pm 1.5\%$, $n = 5$) was Ca^{2+} dependent. It has been suggested that only neuronal and not glial K^+ -induced release of GABA is Ca^{2+} dependent^{13,15}, although there is evidence that the latter may also show some Ca^{2+} dependence¹⁶. Thus, both the high degree of Ca^{2+} dependence of K^+ -induced release in these experiments and the evidence in the literature that cortical uptake (and release) is predominantly neuronal, suggest to us that our results with K^+ stimulation mainly represent effects on efflux from the neuronal compartment.

Muscimol (10^{-7} M) caused a significant reduction in the amount of radioactivity released from the tissue in response to depolarisation with 15 mM K^+ , and this reduction was prevented by either bicuculline or picrotoxin (10^{-6} M) (Fig. 1, Table 1). 3-Aminopropane sulphonic acid also reduced the amount of radioactivity released in the same conditions, as did GABA ($3 \times 10^{-7}\text{ M}$) if fluspirilene (10^{-6} M) was added to the medium to prevent its exchange with radioactive GABA in the tissue¹⁷ (Table 1). Dose-response curves were not determined with the agonists in these experiments because at higher concentrations exchange with tissue ^3H -GABA was observed and at lower concentrations the effects were not statistically significant. The experimental conditions used here apparently prevented the

reuptake of GABA released from the tissue, as the addition of the GABA uptake inhibitor fluspirilene (Janssen, 95% inhibition at 10^{-5} M) did not affect the amount of radioactivity which appeared in the superfusate in basal release conditions. If the reuptake mechanisms were important in these conditions an increase would have been expected. In addition, it was found that fluspirilene (10^{-6} M) was without significant effect on the K^+ -induced release.

Muscimol at 10^{-7} M exhibited no effects on the basal release of GABA, although this compound is a substrate for the high affinity GABA uptake system, and, at 10^{-5} M , displaced GABA from the tissue. None of the other compounds used modified the basal release of radioactivity in the conditions used here.

The evidence presented shows that GABA and the two GABA agonists, muscimol and 3-aminopropane sulphonic acid, reduce the amount of radioactivity released as a result of potassium stimulation and also that the reduction caused by muscimol can be prevented by the GABA antagonists bicuculline and picrotoxin. This is consistent with the idea that the release of GABA from the rat frontal cortex is subjected to a negative feedback control possibly through presynaptic receptors.

In a previous study concerning the effects of various convulsants on electrically induced release of radioactive GABA from cortex slices¹⁸, it was found that 10^{-5} M bicuculline facilitated this release. This is consistent with the above suggestion, although in our experimental situation we found it difficult to demonstrate a facilitation of GABA release by bicuculline or picrotoxin alone at 10^{-6} M or 10^{-5} M . Such a difficulty can be explained if, as we believe, the GABA released in our system is removed by the flow of superfusion fluid before a sufficient concentration develops to stimulate the presynaptic receptors. If this is the case, then the bicuculline and the picrotoxin have no negative feedback effects to antagonise. However, by including a GABA agonist, muscimol, in the superfusion fluid, the presynaptic receptors, by interaction with the agonist, would produce a negative feedback on GABA release which could then be antagonised by bicuculline and picrotoxin.

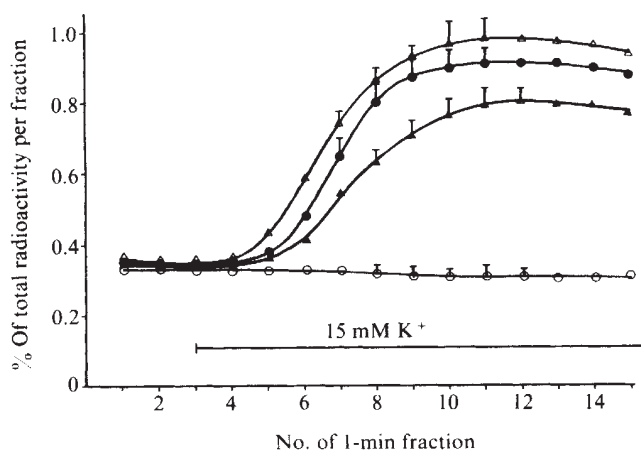
The data presented here with several GABA agonists and antagonists are consistent with the existence of presynaptic receptors on GABAergic terminals capable of controlling the release of this transmitter.

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Fig. 1 The effect of muscimol and muscimol+picrotoxin on potassium-induced release. The horizontal axis represents the number of the 1-min fractions and the vertical axis the radioactivity present in each fraction expressed as a % of the radioactivity in the tissue at the start of the experiment. Each point used in the integration (the first 5 fractions after each curve reaches over 50% of peak height) is shown $\pm$ s.e.m. (where $n = 5$). ●, 15 mM K^+ alone; ▲, $15\text{ mM K}^+ + 10^{-7}\text{ M muscimol}$; △, $15\text{ mM K}^+ + 10^{-7}\text{ M muscimol} + 10^{-6}\text{ M picrotoxin}$; ○, control (normal medium). Substitution of Mg^{2+} for Ca^{2+} in the medium did not significantly affect the basal release, although $80.1 \pm 1.5\%$ ($n = 5$) of the K^+ (15 mM) induced release was dependent on the presence of Ca^{2+} in the medium.



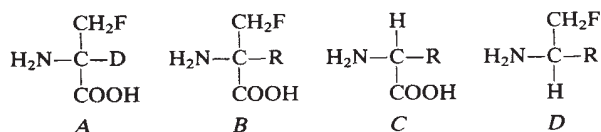
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Selective inhibitors of biosynthesis of aminergic neurotransmitters

ALTHOUGH the enzymatic decarboxylation of amino acids is of substantial importance to biochemistry¹, there are few inhibitors of the decarboxylase enzymes which combine activity with selectivity. Several of the amines formed by *in vivo* decarboxylation of amino acids (biogenic amines) have key roles in physiology. The neurotransmitters dopamine, 5-hydroxytryptamine, histamine and γ -aminobutyric acid result from such enzymatic decarboxylation; dopamine in turn serves as the precursor of noradrenaline². The involvement of catecholamines in peripheral and central control of blood pressure has been the subject of many investigations; for example, elevated catecholamine levels were found in some of the 27 brain regions investigated in spontaneously hypertensive rats. Specifically, elevated noradrenaline and dopamine levels were found in regions implicated in the control of arterial blood pressure³. A widely reported biochemical theory of schizophrenia suggests disturbance of the dopaminergic system as the causative factor.⁴ Elevated histamine levels are believed to be involved in such diseases as allergy, hypersensitivity, gastric ulcer and inflammation⁵. Ornithine decarboxylase is also an important target for inhibition, as it is the initial enzyme in the biosynthesis of polyamines and increased levels of the latter have been associated with rapid cell division, including tumour growth⁶. Thus, selective inhibitors of these key enzymes could be of help in elucidating the complexities of neurophysiology and neurochemistry, as well as of service in medicine by correcting pathological levels of these agonists. We report here examples of the transformation of amino acids (*C*) into the corresponding substituted 3-fluoro-alanines (*B*), resulting in potent time-dependent decarboxylase inactivators (Table 1). In addition, we have prepared the fluoromethyl derivatives (*D*) corresponding to some of the amine products of these decarboxylases and find them also to be inactivators (Table 1).

Consideration of the mechanism of inactivation of the pyridoxal phosphate-dependent bacterial alanine racemase by the experimental antibacterial 3-fluoro-2-deutero-D-alanine (*A*; MK-641)⁷⁻⁹ as an example of suicide substrate inhibition, suggested that properly substituted 3-fluoro-alanines (*B*), that is α -fluoromethylated versions of amino acids (*C*), in turn might be inactivators of another class of enzymes dependent on pyridoxal phosphate—amino acid decarboxylases:



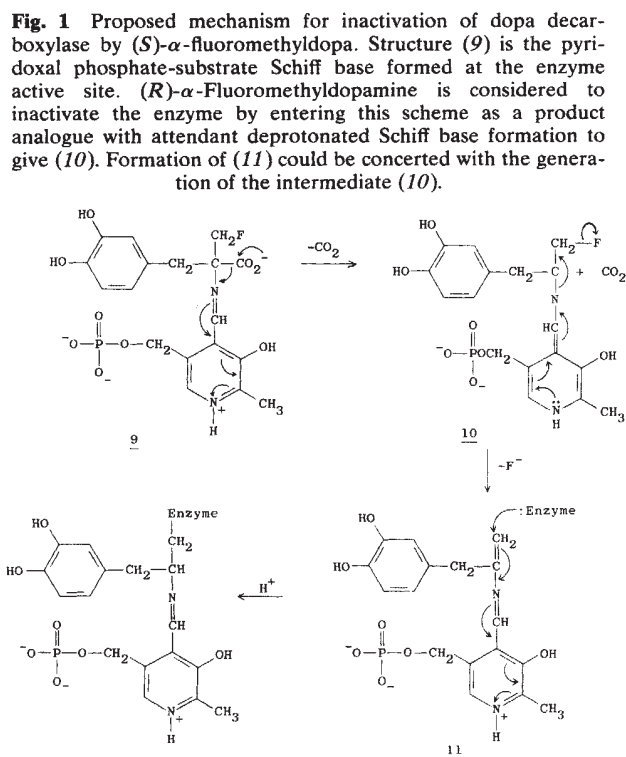
By inactivation is meant irreversible inhibition as indicated by time-dependent loss of enzyme activity. (The deduction that MK-641 is a suicide substrate was recently verified¹⁰. For reviews on suicide substrate inhibitors, see refs 11–13.)

($\pm$)- α -Fluoromethylglutamic acid (*I*) was synthesised by photofluorination¹⁴ of α -methylglutamic acid. The other fluoromethyl amino acids and fluoromethyl amines discussed here were obtained by fluorodehydroxylation¹⁵ of the corresponding α -hydroxymethyl precursors and were characterised by C-H-N-F analysis, ¹H-NMR, ¹⁹F-NMR and optical rotation (where applicable). The copper chelate of ($\pm$)- δ -benzoylornithine was reacted with paraformaldehyde in pyridine-ethanol with K₂CO₃ catalyst. Acid hydrolysis led to ($\pm$)- α -hydroxymethylornithine. (*S*)- α -Fluoromethyldopa (*3*) [$[\alpha]_D$: 9.3 $\pm$ 0.5° (c, 1.82 in trifluoroacetic acid-H₂O 1:1)] was prepared via (*R*)- α -hydroxymethyldopa obtained as follows: 3[3',4'-diacetoxyphenyl]-2-acetamino-2-acetoxymethylpropionic acid was saponified to 3[3',4'-dihydroxyphenyl]-2-acetamino-2-hydroxymethylpropionic acid, which was methylated to 3[3',4'-dimethoxyphenyl]-2-acetamino-2-hydroxy-

methylpropionic acid, which on resolution by strychnine gave the (*R*) acid, [α]_D: 78.3 $\pm$ 0.5° (c, 1.425 in 0.1 M aq. NaOH). Hydrolysis in concentrated HCl gave (*R*)- α -hydroxymethyldopa. (*S*)- α -Fluoromethyltyrosine (*4*) was prepared via the Cu-chelate of ($\pm$)-tyrosine-*O*-methyl ether, which was reacted with paraformaldehyde in pyridine-ethanol with K₂CO₃ catalyst to give 3[4'-methoxyphenyl]-2-amino-2-hydroxymethylpropionic acid. This was *N*-acetylated and resolved with *l* and *d* ephedrine to yield both the (*S*) and (*R*) isomers of 3[4'-methoxyphenyl]-2-acetamino-2-hydroxymethylpropionic acid [$[\alpha]_D$: -81° and 82°, respectively, (c, 1.35 in 0.3 M NaOH)]. Hydrolysis of the (*R*) isomer by concentrated HCl produced (*R*)- α -hydroxymethyltyrosine. The Cu-chelate of *N*_{im}-benzylhistidine was reacted with paraformaldehyde-K₂CO₃ to give α -hydroxymethyl-*N*_{im}-benzylhistidine and the debenzoylation of this with Na/liq. NH₃ led to α -hydroxymethylhistidine. (*R*)- α -Fluoromethyldopamine (*6*) [$[\alpha]_D$: 18.4, c. 11 M HCl] was then prepared by fluorodehydroxylation of (*R*)- α -hydroxymethyldopamine, obtained by reduction of ethyl (*R*) dopate with Ca(BH₄)₂. (*S*)- α -Fluoromethylhistamine (*7*) was prepared by fluorodehydroxylation of histidinol, obtained by reduction of L-histidine¹⁶. (*R*)- α -Fluoromethylhistamine (*8*) was obtained by a similar method, starting with D-histidine. Stereochemical assignment of *3* and *4* was done by circular dichroism.

The activity and specificity of these inhibitors is illustrated by the following. (1) Compared to (*S*)- α -fluoromethyldopa, the (*R*)-antimer is ineffective as an inhibitor or as an inactivator of dopa decarboxylase. (2) (*R,S*)- α -fluoromethylhistidine has little effect on dopa decarboxylase compared to (*S*)- α -fluoromethyldopa, which is a rapid, potent inactivator of this enzyme. (3) Conversely, (*S*)- α -fluoromethyldopa has little effect on mammalian histidine decarboxylase, whereas (*R,S*)- α -fluoromethylhistidine is a potent and rapid inactivator of the enzyme. (4) (*R,S*)- α -fluoromethylhistidine is a potent and rapid inactivator of mammalian histidine decarboxylase, but has little effect on the enzyme from *Lactobacillus* 30A.

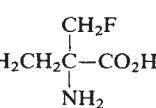
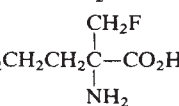
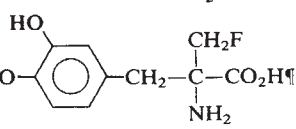
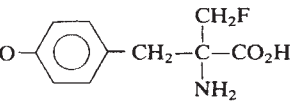
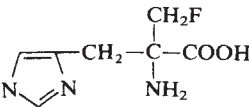
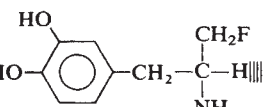
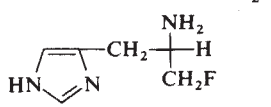
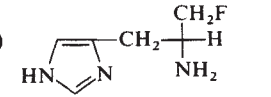
We suggest that the time-dependent character of the inactivation caused by α -fluoromethyl amino acids should be attributed to the fluoroalanyl moiety and the selectivity attributed to the *R* substituents (formula *B*). Time-dependent inhibition (Table 1) suggests irreversible alkylation of the



respective decarboxylases. The inactivation of dopa decarboxylase by (*S*)- α -fluoromethyl-dopa (3) goes to completion with time and is retarded in the presence of the substrate α -methyl-dopa, thereby implicating the active site as the locus of inactivation. No enzyme activity is restored by dialysis. Enzyme inactivated with [ring- ^3H]- α -fluoromethyl-dopa be-

comes associated with approximately one equivalent of tritium whereas enzyme inactivated with [^{14}C -carboxyl]- α -fluoromethyl-dopa incorporates no ^{14}C . Furthermore, the incorporation of tritium is associated with the release of an equivalent amount of fluoride. These data demonstrate the stoichiometric formation of an enzyme-inhibitor adduct

Table 1 *In vitro* activity of α -fluoromethylamino acids and α -fluoromethyl amines, inhibitors of amino acid decarboxylases

Inhibitor Amino acids	Enzyme*	Inhibition data†	Nature of inhibition
1 	Glutamate decarboxylase‡ [EC 4.1.1.15]	K_m (L-glutamate) 9.8×10^{-4} M $K_i > 10^{-3}$ M $t_{50} \sim 180$ min at 3×10^{-3} M	Irreversible
2 	Ornithine decarboxylase§ [EC 4.1.1.17]	K_m (L-ornithine) 2.5×10^{-4} M $K_i 1.8 \times 10^{-5}$ M $t_{50} \sim 5$ h at 2.0×10^{-5} M	Irreversible
3 	Dopa decarboxylase # [EC 4.1.1.26]	K_m (L-dopa) 2×10^{-4} M $K_i 4 \times 10^{-8}$ M t_{50} 4 min at 1×10^{-7} M (in presence of 2.8×10^{-3} M dopa)	Irreversible
	Histidine decarboxylase** (mammalian) [EC 4.1.1.22]	K_m (L-histidine) 1.7×10^{-4} M I_{50} no inhibition at 2.6×10^{-4} M	None observed
4 	Dopa decarboxylase # [EC 4.1.1.26]	K_m (L-tyrosine) 8.4×10^{-3} M # # $K_i > 2 \times 10^{-3}$ M $t_{50} \sim 120$ min at 6×10^{-4} M	Irreversible
5 	Histidine decarboxylase** (mammalian) [EC 4.1.1.22]	K_m (L-histidine) 1.7×10^{-4} M I_{50} 1.3×10^{-5} M†† t_{50} 30 min at 3.8×10^{-6} M	Irreversible
	Histidine decarboxylase§§ (bacterial) [EC 4.1.1.22]	K_m (L-histidine) 1.3×10^{-3} M $K_i > 10^{-2}$ M t_{50} no time dependence at 8×10^{-2} M	None observed
Amines	Dopa decarboxylase # [EC 4.1.1.26]	K_m (L-histidine) high # # $K_i > 10^{-2}$ M t_{50} no time dependence at 1.7×10^{-3} M	None observed
6 	Dopa decarboxylase # [EC 4.1.1.26]	K_m (L-dopa) 2×10^{-4} M K_i n.a. $t_{50} < 10$ min at 1.3×10^{-5} M	Irreversible
7 	Histidine decarboxylase** (mammalian) [EC 4.1.1.22]	K_m (L-histidine) 1.7×10^{-4} M I_{50} 9×10^{-5} M†† t_{50} 30 min at 2×10^{-5} M	Irreversible
8 	Histidine decarboxylase** (mammalian) [EC 4.1.1.22]	K_m (L-histidine) 1.7×10^{-4} M I_{50} 1×10^{-3} M†† t_{50} 30 min at 5×10^{-4} M	Irreversible

* Decarboxylase activity was determined by monitoring²¹ evolution of $^{14}\text{CO}_2$ from the appropriate [^{14}C -carboxyl]amino acid at 37°C. To determine whether a compound caused inactivation, enzyme was preincubated with inhibitor at room temperature in normal assay conditions, but in the absence of substrate. Aliquots were removed and enzyme activity was determined in the normal way.

† K_m and K_i were determined by the method of Lineweaver and Burk²², using initial rates for K_i in those cases in which time-dependent inhibition was rapid. n.a. Not applicable. t_{50} is the preincubation time of enzyme with inhibitor in the absence of substrate which is required to produce 50% inactivation at the specified inhibitor concentration. In most cases, we did not determine whether the inactivation went to completion. I_{50} is the concentration of inhibitor necessary to produce 50% inhibition of the enzyme activity in the specified conditions.

‡ Glutamate decarboxylase was isolated from rat brain by the procedure described for mouse brain²³. Material corresponding to crude extract (specific activity 0.020 units mg^{-1}) was used.

§ Ornithine decarboxylase was isolated from the livers of rats 24 h after injection of thioacetamide²⁴. Enzyme was purified through the acid treatment step (specific activity 0.11 units mg^{-1}).

|| (*R,S*)- α -difluoromethylornithine is reportedly a catalytic irreversible inhibitor of this enzyme²⁵.

¶ The *R*-isomer is ineffective as an inhibitor.

Dopa decarboxylase was isolated from pig kidney²⁶. Enzyme from the second hydroxylapatite column (specific activity 4–9 units μg^{-1}) or from the ammonium sulphate fractionation step (0.05–0.1 units μg^{-1}) was used. The published assay procedure was followed except that we used 2.8 mM L-dopa and 10 or 15 min incubation periods.

** Mammalian histidine decarboxylase was prepared from 19-d fetal rat liver²⁷ (0.02–0.04 units mg^{-1}) and assayed at 37°C for 40 min with 0.1 M phosphate buffer, pH 7.0, 2×10^{-4} M L-histidine, 3.4×10^{-5} M pyridoxal phosphate and 60–70 μg protein in a total volume of 1.0 ml.

†† Determined in normal assay conditions.

At pH 8.5. Value taken from ref. 26.

§§ A gift from Dr James Steffens; purified²⁸ from *Lactobacillus* 30A.

||| The *S*-isomer binds to the enzyme but causes no inactivation (30% inhibition at 3.9×10^{-4} M).

which lacks both the carboxyl and the fluoride of the inhibitor.

Since 3 does not seem to be intrinsically reactive in nonequilibrium conditions (stable in 1 M NaOH at room temperature for 24 h), its inhibitory activity depends on the enzyme's ability to catalyse the conversion of it to a reactive species. Taken together, our data classify (S)- α -fluoromethyl-dopa as a suicide inactivator of dopa decarboxylase. A plausible mechanism of inactivation is outlined in Fig. 1. (Note that the extraordinary selectivity of suicide substrate enzyme inactivators is discussed in refs 11–13.)

α -Fluoromethylation of dopa to give 3 demonstrates the first instance of transformation of an amino acid substrate into suicide enzyme inactivator of the respective decarboxylase enzyme. (In contrast, α -vinylation or α -ethynylation of dopa led to partial inhibitors of dopa decarboxylase^{17,18}; most of this inhibition is reversible on dialysis¹⁹. The first example for the transformation of a product of enzymatic decarboxylation of an amino acid into suicide inhibitor of the respective decarboxylase was provided by the synthesis of γ -ethynyl-GABA, an inhibitor of glutamic acid decarboxylase²⁰.) By substituting other haloalkyl groups in place of the fluoromethyl group α -difluoromethyl-dopa, α -difluoromethyl-dopamine and α -trifluoromethyl-dopamine were synthesised and found to be inactivators of dopa decarboxylase (details to be published). In addition, (S)- α -fluoromethyl-dopa (3) and (R,S)- α -fluoromethylhistidine (5) were found to powerfully inhibit *in vivo* dopa decarboxylase and histidine decarboxylase, respectively²⁹.

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A circulating variant of human proalbumin

ALBUMIN is initially synthesised in the liver as preproalbumin¹. This has an N-terminal peptide which is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to give the secreted albumin². We report here the finding of a family with a circulating variant of proalbumin. This provides information on the structure of human proalbumin and the specificity of the propeptide cleavage site. It also raises the problem of the biological function of the propeptide and the necessity for cleavage.

An abnormal albumin was noted in seven individuals during diagnostic serum protein electrophoresis. Further investigation showed that these individuals, all of European descent, represented four generations of one family with ages ranging from 73 yr to early childhood. A review of their case histories confirmed the impression, from their age distribution, that the albumin abnormality was not associated with discernible handicap.

The variant albumin was isolated from 25 ml of serum by adding powdered polyethylene glycol 6000 to give a concentration of 16% (w/v). The suspension was stirred for one hour in the cold before being centrifuged. The variant albumin was isolated by chromatography of the supernatant on DEAE Sephadex using 25 mM acetate buffer with a 5.1–4.5 pH gradient. S-Carboxymethylation of the cysteine residues was carried out in 8 M urea³. Tryptic digestion, peptide mapping and peptide analysis were carried out essentially as described by Chua *et al.*⁴. Digestion with *Staphylococcus aureus* V8 protease (Miles) was carried out in 0.2 M ammonium bicarbonate for 16 h at 37 °C. The albumin concentration was 10 mg ml⁻¹ and the protease substrate ratio 1:300. Peptides were sequenced by the dansyl-Edman technique⁵ and N-terminal sequence analysis of the protein was carried out in dodecyl sulphate⁶.

The chromatographically isolated variant (Fig. 1) formed 50% of the total albumin. The initial amino acid sequence of both the normal and abnormal components was determined as summarised in Fig. 2. The abnormality was indicated from the tryptic peptide maps which showed that the normal N-terminal tryptic peptide (Tr A1) was replaced by two new peptides (Tr X1 and Tr X2). The sequence of these peptides and an initial N-terminal sequence analysis of the protein showed that the abnormality was due to the presence of an additional N-terminal sequence, Arg-Gly-Val-Phe-Arg-Gln. Comparison of this with the propeptides of rat⁷ and bovine⁸ proalbumin, both Arg-Gly-Val-Phe-Arg-Arg, indicated that the abnormal protein was a variant of proalbumin with, by inference, a point mutation of the terminal propeptide residue to glutamine.

The finding of proalbumin Christchurch, as it has been named, demonstrates for the first time the structure of human proalbumin. It also confirms that a pair of basic residues is a prerequisite for propeptide cleavage. This had been assumed from the consistent finding of paired basic residues in propeptides in general⁹ and, in particular, it had been concluded that proalbumin cleavage was due to a specific protease released within the Golgi vesicles². However, the finding¹⁰ that chicken proalbumin has only one arginine has raised the possibility that propeptide cleavage may be due to nonspecific tryptic-like proteolysis. If this were a general mechanism, then cleavage should still occur at the remaining arginine residue of proalbumin Christchurch. The failure of this cleavage confirms that a specific protease is involved, probably² cathepsin B.

The finding that the proalbumin was present in the plasma in equal proportions with its normal allele shows that the cleavage of the propeptide is incidental to the process of secretion. Also, the fact that overall albumin levels were not raised is evidence against the proposal¹¹ that the released propeptide acts as a

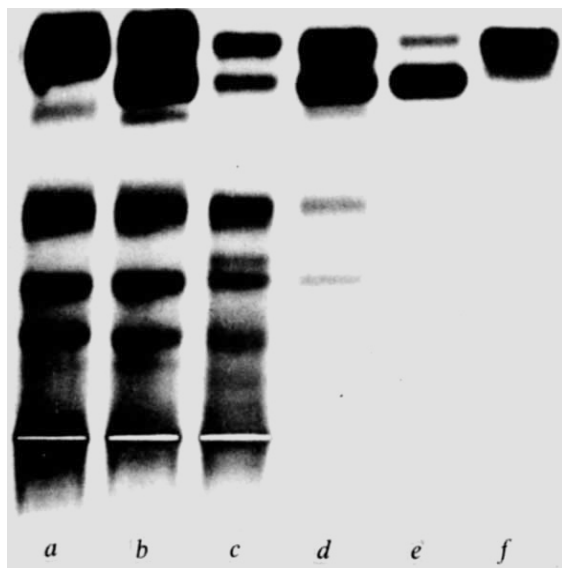


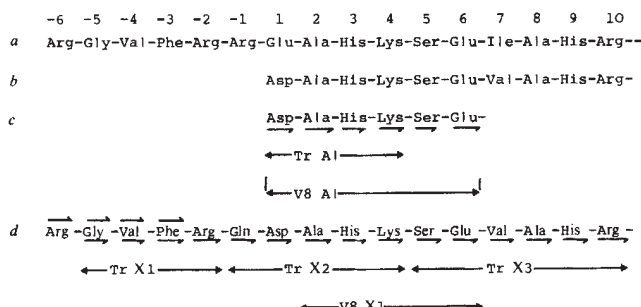
Fig. 1 Agarose gel electrophoresis, pH 8.6, anode at top. *a*, Normal serum; *b* heterozygous carrier of proalbumin Christchurch; *c*, 16% polyethylene glycol precipitate; *d*, supernatant from *c*; *e*, *f*, major peaks from DEAE-Sephadex chromatography.

feedback inhibitor of synthesis. If this were so, the failure to release 50% of the propeptide would be expected to result in a discernible increase in plasma albumin.

Functionally, the presence of the propeptide should have some effect for it will block the high affinity binding site of Cu(II). Albumin is the major transport protein for readily exchangeable Cu(II); this is bound¹² as a ternary coordination complex by the three N-terminal residues, the critical requirement being a histidine at position 3. The loss of this binding site from 50% of the albumin in the heterozygote for proalbumin Christchurch should not cause a major hardship; indeed, the dog, which has a tyrosine in position 3, functions without an albumin copper-carrying site¹³.

What, then, is the function of the propeptide? Is it an evolutionary remnant or a protein punctuation mark, or does it have an essential functional role? The findings from this family study would indicate that it does not affect the secretion, absorption or gross function of the protein. However, it will require the finding of a homozygote to discover if the retention of the propeptide provides a more subtle handicap or whether the cleaved propeptide, itself, has some essential function.

Fig. 2 Amino-terminal sequences of *a*, rat proalbumin⁷; *b*, human albumin¹⁴; *c*, patient's normal albumin; *d*, proalbumin Christchurch. The sequenced residues of peptides are shown by →, protein sequence data by —. Cleavage positions are shown for trypsin (Tr) and *S. aureus* V8 protease (V8).



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Trypsin-binding IgG in cystic fibrosis

CYSTIC FIBROSIS (CF) is an autosomal recessive genetic disorder with a frequency of about 1/1,600 among newborn infants of European descent. In spite of extensive investigation its primary molecular abnormality remains unknown. It has been observed that most CF patients lack an α_2 macroglobulin-trypsin complex¹ and that binding of trypsin and other proteases to α_2 macroglobulin (α_2 M) is lower in plasma of CF patients than in that of normal controls². We therefore decided to study the interaction of trypsin with plasma proteins of CF patients. Here we report that trypsin-binding IgG (TbIgG) is present in 80% of CF patients and in 30% of their mothers, but not in 97% of normal controls or in other relatives of CF patients. Experimental evidence supports the antibody nature of TbIgG, which is presumably produced against the porcine trypsin ingested by CF patients as pancreatic extracts for substitutive enzyme therapy.

Samples of plasma or serum were incubated with porcine or bovine ¹²⁵I-trypsin, subjected to polyacrylamide gel electrophoresis (PAGE) and autoradiographed as described in Fig. 1. The multiple bands in the upper portion of the gel were identified as α_2 M-trypsin complexes by immunoelectrophoresis on PAGE, using monospecific antiserum against human α_2 M (Atlantic Antibodies). These bands were not observed when trypsin was preincubated with an irreversible inhibitor such as tosyl-lysyl-chloromethyl-ketone (TLCK) or when the plasma was preincubated with antiserum against α_2 M. No difference was found between CF patients and controls with respect to these bands; it is now being determined whether they represent α_2 macroglobulin-trypsin complexes of different molecular weight, breakdown products or other molecular forms.

An unexpected difference between CF patients and controls was observed. As Fig. 1 shows, the fast moving trypsin bands were clearly preserved in the presence of sera or plasma from normal individuals but were missing when trypsin was mixed with sera or plasma of CF patients. In the latter samples, however, radioactive material appeared in the γ -globulin region, that is, toward the cathodal end of the gel. Immunoelectrophoresis in agar-gel of ¹²⁵I-trypsin incubated with sera of control individuals and of CF patients, followed by autoradiography (Fig. 2) clearly confirmed that the 'missing' trypsin bands in CF samples are actually bound to IgG. We noticed in a few patients that IgA also binds trypsin, but less regularly than IgG. We therefore called these immunoglobulins trypsin-binding IgG or TbIgG. TbIgG can also bind trypsin inhibited by TLCK or soybean trypsin inhibitor.

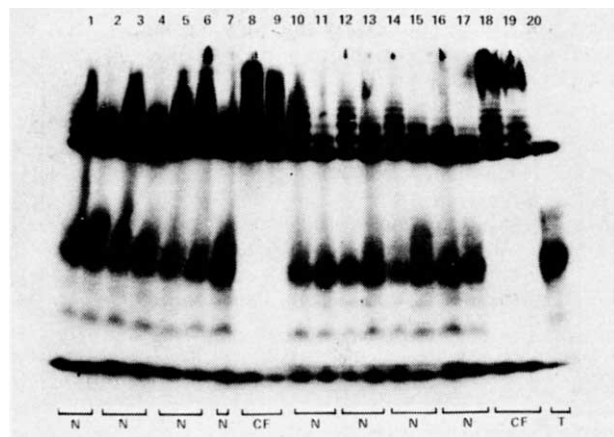


Fig. 1 PAGE followed by autoradiography of sera and plasma samples from normal individuals and CF patients. Samples of 20 μ l of serum or plasma were incubated for 30 min at 37 °C with 5 μ l (25,000 c.p.m.) of porcine trypsin (Worthington) labelled with 125 I (3) which had a specific activity of 20 mCi mg $^{-1}$. Samples of 15 μ l were loaded on the gel, electrophoresed and autoradiographed for 4 d (ref. 4). Wells 1 to 7 and 10 to 17 contained sera or plasma from controls. Wells 8 and 9 contained serum and plasma from a CF patient and 18 and 19 from another CF patient. Radioiodinated trypsin alone (in well 20) showed several electrophoretic bands, probably due to a combination of autodigestion and breakdown caused by iodination.

To understand which portion of the IgG molecule is responsible for binding trypsin, CF and normal IgG were digested with papain. Papain cleaves the IgG molecule into a Fc fragment and two monovalent Fab fragments containing identical antigen-combining sites⁵. Only CF Fab fragments were capable of binding trypsin; no binding to Fc fragments from normal or CF IgG was observed (Fig. 2). These results indicate that TbIgG represents antibodies against trypsin. Whether the antigen responsible for the presence of these antibodies is only the exogenous trypsin or also the endogenous human enzyme remains to be established.

Immunoprecipitation of IgG from CF patients and normal controls after incubation with trace amounts of 125 I-trypsin confirmed the results obtained by PAGE analysis. Therefore, we have used PAGE analysis to screen 302 individuals, including 53 CF patients, 90 parents and siblings of patients and 159 normal controls. All CF patients who had TbIgG (80%) had a positive history of trypsin intake. The correlation between trypsin intake and presence of TbIgG is very high (by Fisher's exact test) (Table 1). The average age of the patients negative for TbIgG is higher and the clinical scores suggest that they are more mildly affected than those positive for TbIgG. In this respect it is interesting that pancreatic damage is absent even as late as 10 yr of age in about 20% of the CF patients, while in 80% the pancreatic disease process probably begins very early in neonatal or fetal life⁶.

Screening of parents and siblings of CF patients for TbIgG revealed that 30% of the mothers were positive, while 30 fathers and 19 siblings were all negative (Table 2). Because powdered pancreatic extracts have been reported to elicit allergic responses in parents of CF children^{7,8} we asked all

subjects about exposure to such preparations. Of the 11 mothers who were positive, six recollected being exposed to powdered pancreatic extracts. The remaining five mothers could have been exposed to pancreas preparations by handling uncoated tablets of pancreatic extracts which are taken daily by CF patients. Interestingly, the histories of exposure to pancreatic extracts offer an explanation for our findings in two out of four individuals positive for TbIgG among 159 normal controls tested. Both of these individuals had been in close contact with CF patients, one being a nurse who works with CF children and the other the husband of a CF woman.

Finally, two more adults affected with chronic pancreatitis (blood provided by Dr M. Vogel of Sunnyvale Medical Center, California) were included in our study. These patients, who had been taking pancreatic extracts for more than 1 yr, were positive for TbIgG by PAGE and by immunoprecipitation analyses.

The correlation between the presence of TbIgG in CF patients and a history of intake of pancreatic extracts leads to the hypothesis that exposure to exogenous trypsin is a necessary condition for the development of TbIgG. Because pancreatic involvement is a prerequisite for replacement enzyme therapy we find TbIgG in those patients with substantial pancreatic damage. It has been shown that in rats and human subjects treated with oral antibiotics the levels of species-homologous immunoreactive trypsin and elastase increase considerably in faeces (ref. 9 and unpublished results of S. Genell and X. Gustafsson). Human elastase labelled with 131 I administered into the duodenum has been recovered bound to α_1 antitrypsin and α_2 M in plasma of one out of eight individuals¹⁰. An enteropancreatic circulation of digestive enzymes has also been proposed as a physiological model in the rabbit, but has not been demonstrated in man¹¹. Intact digestive enzymes might therefore be absorbed through the intestinal mucosa in

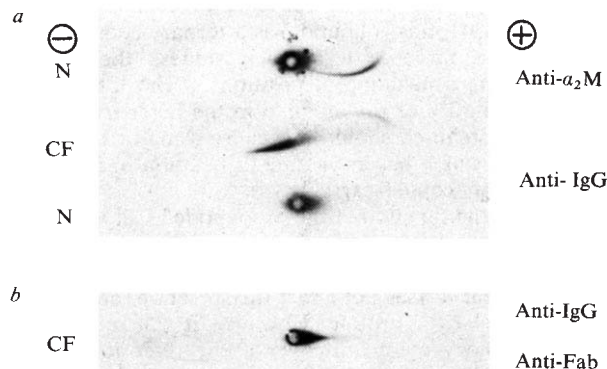


Fig. 2 *a*, Demonstration of TbIgG by immunoelectrophoresis and autoradiography. Samples of 3 μ l of sera from a CF patient and a normal control, incubated with 125 I as described for Fig. 1, were electrophoresed in agar gel⁵. 125 I-trypsin binds to α_2 macroglobulin (α_2 M) as shown by the radioactive arc visible after precipitation with anti- α_2 M antiserum. In serum of CF patients a substantial amount of radioactive trypsin migrated in the γ -globulin region, towards the cathode, as shown by the radioactive arc visible after precipitation with anti-human IgG. This radioactive arc was not visible with sera from controls. *b*, Papain digestion of CF and normal IgG. CF and normal sera were fractionated by ammonium sulphate precipitation and the IgG fractions were digested with papain⁵, which is known to cleave the IgG molecule to yield two monovalent Fab fragments containing the antigen-combining sites and an Fc fragment. The resulting mixture of Fab and Fc fragments were incubated with 125 I-trypsin and subjected to immunoelectrophoresis as described above. The antiserum against Fab was applied in the lower trough and that against IgG (γ chain specific which reacts with the Fc portion) in the upper trough. Autoradiography of the agar gel showed that only the CF-derived Fab fragment bound 125 I-trypsin. 125 I-trypsin inhibited with soybean trypsin inhibitor also bound CF Fab fragments; no binding to Fc fragments of normal or CF IgG was found (not shown).

Table 1 Screening of 53 CF patients by PAGE

	Total (%)	Mean age ($\pm$ s.e.)	History of trypsin intake		Sex	
			+	-	Male	Female
Positive	42 (80)	14.5 ($\pm$ 6.5)	42	0	22	20
Negative	11 (20)	22.9 ($\pm$ 2.2)	6	5	4	7

Table 2 Screening of CF relatives and controls by PAGE

	Fathers	Mothers	Sibs	Controls
Positive	0	11	0	4
Exposed to pancreas Powder?		Yes 6 No 5		Yes 2 No 2
Negative	30	30	19	155
Exposed to pancreas Powder?	Yes 13 No 9 Not asked 8	10 10 10	5 10 4	5 66 84
Totals	30	41	19	159

certain conditions, such as oral antibiotic treatment, which occur in most CF patients. On the other hand, the presence of TbIgG in mothers of CF patients and especially in those who do not recollect any exposure to powdered pancreatic extracts is surprising. Experiments are in progress to determine whether CF patients and their mothers possess antibodies directed against human trypsin as well as other endoproteases: antibodies against porcine elastase but not against papain or beef thrombin have been found in some CF patients.

The level of immunoreactive trypsin (trypsin and trypsinogen being indistinguishable by radioimmunoassay techniques; M. C. Geokas, personal communication) is considerably decreased in patients affected with chronic pancreatitis¹². Our demonstration of TbIgG in two such patients suggests that antibodies may be responsible. It is quite likely that circulating trypsin and trypsinogen are bound *in vivo* by TbIgG present in sera of chronic pancreatitis patients and CF patients.

Rabbit antibodies against trypsin inhibit the activity of the enzyme towards both high and low molecular weight substrates¹³. Their affinity for trypsin increases with prolonged immunisation and is proportional to their inhibitory capacity¹⁴. A decrease in trypsin-like or arginine esterase activity which can be inhibited by soybean trypsin inhibitor has been described in plasma of CF patients and some heterozygotes¹⁵⁻¹⁷. The presence of anti-protease antibodies may have a bearing on the lowered arginine esterase activity observed in these studies. On the other hand, other authors have not found a decrease of arginine esterase in CF patients¹⁸⁻²⁰.

Using the liquid phase radioimmunoprecipitation assay described by Shapira *et al.*², we have found²¹ that: (1) TbIgG binds significant amounts of trypsin at trypsin concentrations 10 times lower than that for optimal trypsin binding to α_2 M; (2) TbIgG does not interfere with trypsin binding to α_2 M at high trypsin concentrations (approaching or at saturation of trypsin for α_2 M), and (3) there is no difference in trypsin-binding between CF and normal α_2 M.

Several important questions remain. Are there antibodies directed against human trypsin? What is the extent of the immune reaction in the mucosa? Are proteases bound to TbIgG fully inhibited or are they capable of limited proteolysis *in vivo*? We have shown that chronic oral enzyme therapy results in the formation of antibody directed against the therapeutic agent itself. The effect of such an immune response on the efficacy of treatment is not yet known, but is clearly relevant to numerous other diseases in which chronic oral therapy is required.

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Direct analysis of the chromosome constitution of human spermatozoa

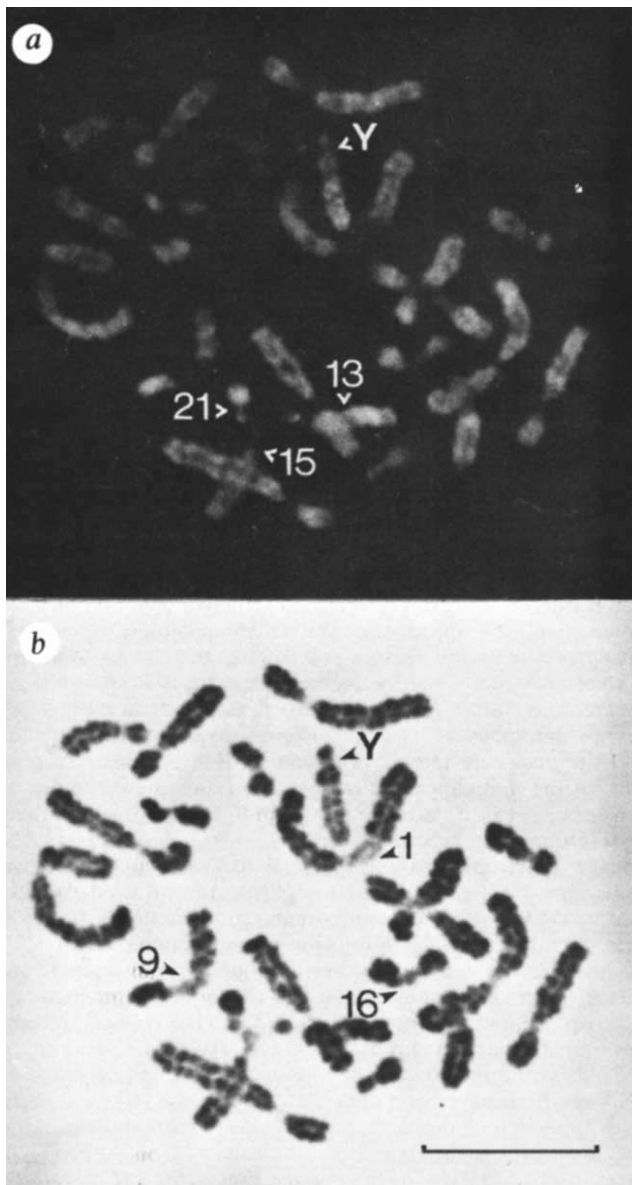
ALL available information on the chromosome constitution of human gametes is speculative, having been obtained by inference from the chromosome constitution of conceptuses that survive sufficiently long to produce a clinically recognisable pregnancy. A minimum of 10% of all recognised human conceptions are chromosomally abnormal, and it has been estimated that 1-2% are the result of fertilisation by a spermatozoon with a chromosome abnormality¹. Cytological evaluation of the chromosome constitution of human spermatozoa has been restricted to the staining of fixed smears of whole sperm²⁻¹³. Certain chromosome regions with peculiar staining properties, such as the long arm of the Y chromosome and the heterochromatic region of chromosome 9, are presumed to be represented in appropriately stained sperm nuclei by differentially staining spots. By counting the number of these spots per nucleus, the frequency of aneuploidy in the sperm of normal males has been estimated to be around 40% (refs 5, 9-11). The precision of the data obtained from stained whole sperm is dubious, because several factors must be taken into consideration when blobs are counted in sperm head nuclei, all of which could contribute to biased estimates of nondisjunction^{7,8,12}. For this reason, some claims have now been retracted^{12,13}. To investigate the true contribution of male gametes to the production of chromosomally abnormal conceptuses and the factors influencing the production and survival of chromosomally abnormal sperm, it is necessary to analyse the sperm chromosomes directly. However, after meiotic metaphase II sperm chromosomes do not reappear until the male and female pronuclei of the fertilised egg prepare for the first cleavage division. We report here the use of hamster eggs to activate human sperm to the point where their chromosomes can be studied directly.

Several attempts have been made to reactivate the sperm nucleus by the fusion of spermatozoa with cultured somatic cells *in vitro*¹⁴⁻¹⁷; this fusion occurs spontaneously and also after treatment with lysolecithin and Sendai virus^{18,19}. However, fusion with cultured cells generally fails to reactivate mature spermatozoa, possibly due to the lack of some factor(s) usually present in the oocyte which induces the decondensation of the tightly packed chromatin, necessary for the resumption of DNA synthesis. Even in the cases where small amounts of DNA synthesis have been observed in heterokaryons^{17,19}, there have been no indications of a subsequent mitosis. The environment provided by the cytoplasm of a mature oocyte seems to be necessary for the reactivation of the sperm nucleus

and subsequent decondensation of the chromatin. The use of hamster eggs as 'reactivating vehicles' for human sperm was first described by Yanagimachi *et al.*²⁰. The technique was devised to evaluate capacitation of human sperm assessed by their ability to penetrate zona-free hamster eggs with subsequent nuclear swelling.

The medium used in all our experiments is a modified Krebs-Ringer's solution developed by Biggers, Whitten and Whittingham²¹, hereafter referred to as BWB. The BWB medium

Fig. 1 The chromosomes of a human spermatozoon with a 23,Y complement. The penetrated hamster eggs were hypotonically treated with 0.1% sodium citrate for 10–20 min and fixed with 3:1 (3 parts absolute methanol: 1 part glacial acetic acid) according to the method described by Tarkowski²³. Slides were stained with Atebrin/quinacrine mustard (0.46 g Atebrin + 0.04 g quinacrine mustard per 100 ml McIlvaine buffer, pH 5.0), mounted in the same buffer and examined with a Leitz Ortholux microscope with a 200 W/4 mercury lamp, a BG12 exciter filter and a 510 nm barrier filter. The chromosomes are easily identifiable when examined on fluorescence because they show the Q-banding pattern characteristic of human chromosomes (a). The slides were subsequently stained with lactic aceto-orcin, dehydrated in xylene and mounted in DePeX (b). Note the heterochromatic regions of chromosomes 1, 9, 16 and the Y and the satellite associations between chromosomes 13, 15 and 21. Scale bar = 10 μ m.



we used contained 0.3% human serum albumin (Pentex, fraction V; Miles) and 1.71 mM CaCl_2 instead of Ca-lactate, and had a pH value of 7.4–7.5 when equilibrated with 5% CO_2 in air.

The semen sample to be tested was collected in a sterile airtight jar, kept at 37°C for about 20 min until the semen liquefied, and then diluted with about 8 ml of BWB and filtered through two layers of sterile 'Kimwipes' paper tissues. The sample was centrifuged in a sterile plastic tube at 600g for 6 min and the sperm pellet washed and centrifuged twice more, then resuspended in 1–2 ml of BWB. A 0.2-ml droplet of BWB was placed in the centre of a sterile plastic Petri dish (3.5 × 1.0 cm) and covered with mineral oil. Two to four drops of the final sperm suspension were dropped from a Pasteur pipette onto the surface of the oil and mixed with the BWB droplet. The final sperm concentration per dish was approximately $3 \times 10^7 \text{ ml}^{-1}$. The dishes were incubated at 37°C in 5% CO_2 in air for 5–7 h to effect capacitation.

To obtain a large number of eggs, superovulation was induced in adult female golden hamsters (*Mesocricetus auratus*) by intraperitoneal injection of 25 IU pregnant mares' serum gonadotrophin on day 1 (the day of ovulation) of the animals' oestrous cycle, followed by an intraperitoneal injection of 25 IU human chorionic gonadotrophin (HCG) on day 3. The animals were killed 17 h after the HCG injection, their oviducts dissected out and the cumulus mass containing the ova removed. The cumulus cells were removed with 0.1% hyaluronidase in BWB at room temperature for 10–15 min, the eggs washed twice in BWB, and the zonae pellucidae digested off with 0.1% trypsin in BWB at room temperature. The zona-free eggs were washed in BWB then transferred to a 0.2-ml droplet of BWB under mineral oil in a sterile plastic Petri dish. Approximately 40–50 eggs were recovered from each superovulated female hamster. We regularly used eggs from four hamsters to test each semen sample.

To effect sperm penetration, two to three drops of the pre-incubated sperm suspension were dropped from a Pasteur pipette onto the surface of the oil in each dish and mixed with the BWB droplet containing the eggs. The dishes containing eggs and sperm were incubated at 37°C in 5% CO_2 in air for 3 h, after which at least 75% of the eggs have been penetrated by one or more sperm. The eggs were placed in a fresh 0.2-ml droplet of BWB under mineral oil and reincubated for 12–13 h. They were then transferred to dishes containing a 0.2-ml droplet of $0.5 \mu\text{g ml}^{-1}$ colcemid in BWB under mineral oil, incubated for a further 6–7 h, then fixed (see figure legends for details) and examined by phase contrast microscopy. Fixed preparations were either stained with an Atebrin/quinacrine mustard solution and subsequently stained with lactic aceto-orcin (Fig. 1), or C-banded (Fig. 2).

Approximately 75% of the eggs which had been incubated with sperm reached the fixation stage, most of the losses having been due to egg lysis. Of the fixed eggs, slightly more than half contained discrete haploid sets of both hamster and human metaphase chromosomes, with up to three sperm complements per egg. On average, we could analyse a maximum of 10 sets of sperm chromosomes each time a semen sample was tested. There were an additional 20–40 sperm complements which were not analysed due to insufficient spreading of the chromosomes.

We have used semen samples from a healthy, 23-yr-old male with a large Y chromosome almost the size of one of the D group chromosomes. The sperm chromosomes are large, with parallel chromatids in which distinctive coils can be seen. Furthermore, in some of the preparations, the heterochromatic regions of chromosomes 1, 9, 16 and the Y chromosome seem to be differentially less condensed than the rest of the chromatin. This is particularly evident in some C-banded preparations where the deeply staining regions of these four chromosomes are seen as disproportionately large by comparison with their appearance in dividing lymphocytes. In some preparations, satellite associations involving the nucleolus organiser loci of

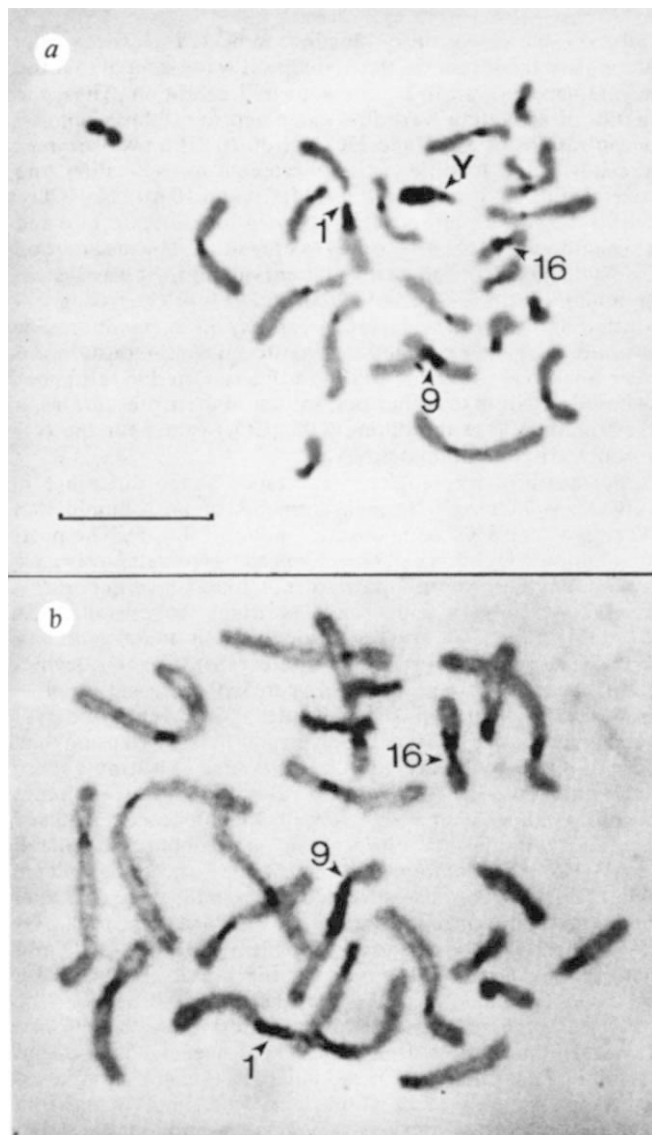


Fig. 2 Two human spermatozoa C-banded by the method of Sumner²⁴. One (a) has a 23,Y complement and one (b) has a 23,X complement. Note the elongated C-bands of chromosomes 1, 9 and 16. Scale bar = 10 μ m.

the acrocentric chromosomes can be seen. This probably reflects nonrandom associations of nucleolar organising regions during or before the onset of ribosomal RNA synthesis. It has been shown that ribosomal RNA synthesis is one of the first processes to be resumed in the fertilised mouse egg²².

Of the 60 sperm we have analysed, 31 have a 23,X constitution, 26 a 23,Y constitution, and 3 are aneuploid, with 22,X,-G, 22,X,-F, and 24,X,+mar,+ace complements. The frequency of aneuploidy in this small sample is therefore 5%.

This technique gives excellent cytological preparations of sperm chromosomes which can, for the first time, be analysed with the same precision as the chromosomes of somatic cells. Assuming that the chromosome constitution of human sperm does not affect their ability to penetrate hamster eggs, a reasonable assumption in view of the known lack of phenotypic expression of the genotype of male gametes, the way is now open to studying directly the chromosome constitution of a population of sperm from any given semen sample and to assessing the effects of various natural and experimentally induced phenomena on the chromosomes of mammalian gametes.

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Control of photorespiration at RuBP carboxylase/oxygenase level in ryegrass cultivars

PHOTORESPIRATION is probably a key intrinsic factor limiting photosynthetic productivity in C_3 plants. Attempts to control photorespiration with inhibitors of the metabolism of various photorespiratory intermediates have yielded unsatisfactory results with whole plants. A logical endogenous control point would be the branch point between photosynthetic and photorespiratory pathways, as most if not all the substrate for photorespiration is formed by the oxygenase function of ribulose biphosphate carboxylase (RuBP carboxylase). There is considerable direct and indirect evidence suggesting that the oxygenase and carboxylase functions of this enzyme are tightly linked^{1,2}. However, temperature has been shown to have differential effects on the affinities of soybean RuBP carboxylase for CO_2 and O_2 (ref. 3), and mutant strains of *Chlamydomonas* and tobacco with altered carboxylase/oxygenase affinities have also been reported^{4,5}. We present here evidence for genetically controlled variation in the relative affinities of the RuBP carboxylase from ryegrass for CO_2 and O_2 .

The enzyme was extracted from cultivars which included both Italian and perennial ryegrass, a range of maturity types and diploid and tetraploid material. Two isozymes were found in purified extracts subjected to isoelectric focusing in polyacrylamide gel (Fig. 1). There is an obvious correlation between isozyme and ploidy among the six commercial cultivars shown and as this is reproduced in the isogenic diploid and tetraploid lines, it seems likely that the difference between the two isozymes can be ascribed to ploidy *per se*. Moreover, when crude homogenates of diploid and tetraploid material were mixed before purification and isoelectric focusing, the expected amounts of the two purified isozymes were obtained, indicating that their occurrence is unlikely to be artefactual.

Kinetic studies indicate that the isozymes have functional significance, as they differ in their affinity for CO_2 but not in that for O_2 in both carboxylase and oxygenase assay systems. However, the V_{max} for the carboxylation reaction is apparently unaltered (Table 1). Lineweaver-Burk and Dixon plots for O_2

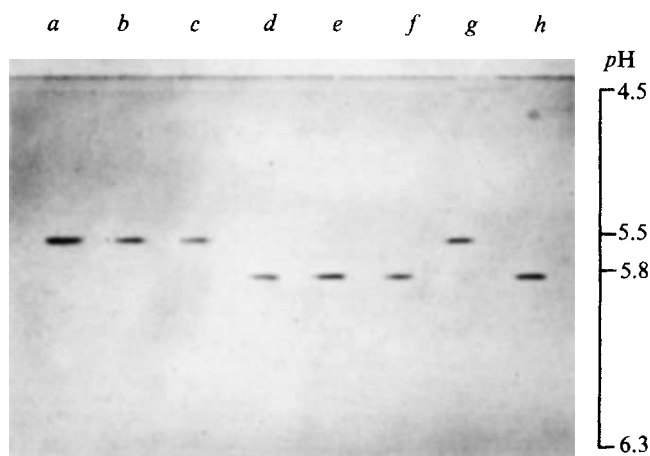


Fig. 1 Isozymes of RuBP carboxylase/oxygenase (EC 4.1.1.39) from various cultivars of perennial and Italian ryegrass (*Lolium perenne* L. and *Lolium multiflorum* Lam., respectively). Cultivars: a, Gremie (diploid perennial); b, Perma (diploid perennial); c, RvP (diploid Italian); d, Barlatra (tetraploid perennial); e, Reveille (tetraploid perennial); f, Tetila Tetrone (tetraploid Italian); g and h, isogenic diploid perennial and tetraploid perennial, respectively. Isoelectric focusing was carried out in polyacrylamide gel (LKB Multiphor system). The pH gradient was determined with a surface electrode. RuBP carboxylase activity assayed in eluates of thin gel slices was associated only with the bands which are shown here stained with Coomassie brilliant blue. The RuBP carboxylase isozymes were extracted from ryegrass as follows: 20 g of youngest fully expanded leaves were homogenised in 30 ml of 40 mM Tris-HCl, pH 8.0, containing 10 mM MgCl₂, 5 mM dithiothreitol and 0.25 mM Na EDTA. The homogenate was filtered through four layers of cheesecloth, and carboxylase protein precipitated in a 37–52% saturated (NH₄)₂SO₄ cut and recovered by centrifugation (30,000g, 10 min). The protein was dissolved in 25 mM Tris-HCl, pH 7.4, containing 10 mM MgCl₂ and 5 mM dithiothreitol, dialysed against the same buffer, then concentrated using an Amicon XM 300 membrane. The concentrate was fractionated in the same buffer on a Sepharose CL-6B-200 gel column (50 cm × 1 cm), eluted protein in the molecular weight range 500,000–590,000 being refractionated on the same column to produce a discrete peak corresponding to a molecular weight of 550,000. This peak was the material used in all isoelectric focusing and enzyme kinetic studies. The purification procedure was carried out without interruption at 6 °C.

inhibition of carboxylase function indicated classical fully competitive inhibition. In these studies it was essential that the enzyme was assayed in a fully activated condition. Thus, the kinetics of activation were first examined to establish optimal concentrations of Mg²⁺ and HCO₃⁻ (ref. 6). The two isozymes were not found to differ in requirements for activation, the process being saturated at 20 mM MgCl₂ and 10 mM NaHCO₃, nor did they differ in their pH optima in both carboxylase and oxygenase assays (pH 8.1). Assays were initiated by the addition of a small aliquot of fully activated enzyme to the assay system containing substrate levels of RuBP and CO₂ or O₂. No lag was detected and the time courses of carboxylation (monitored at 10-s intervals) and oxygenation (monitored continuously) were linear for at least 1.5 min, and so results reported in this paper are based on 1-min assay periods. In view of these precautions, it seems unlikely that the different *K_m*(CO₂) values for the two isozymes arise methodologically.

The possible physiological significance of the difference in *K_m*(CO₂) is indicated by measurements of post-illumination CO₂ burst⁷ and CO₂ compensation point (Table 1). The post-illumination CO₂ burst can be influenced by stomatal resistance but diploids and tetraploids were not found to differ in this respect over a very wide range of diffusion resistance (3–26 s cm⁻¹). The CO₂ compensation point is independent of stomatal resistance. Thus, it is of interest that the two isozymes of RuBP carboxylase seem to correlate with different levels of photorespiration in diploids and tetraploids. Tetraploid ryegrasses have larger tillers and higher rates of leaf extension than those of diploid cultivars¹² and it is tempting to attribute these characteristics to the relatively low rates of photorespiration of the tetraploid. Randall *et al.*¹³ showed that differences in rates of net photosynthesis between decaploid and hexaploid lines of tall fescue (*Festuca arundinacea* Schreb.) were correlated with a higher RuBP carboxylase-specific activity in the decaploid line, but photorespiration, expressed as a percentage of the total CO₂ fixed on both a leaf area and a specific leaf weight basis, did not change significantly with ploidy. In the present study, RuBP carboxylase protein was assayed by quantitative absorption with RuBP carboxylase antisera raised against both the purified diploid and tetraploid isozymes. The isogenic diploid and tetraploid lines did not differ significantly in the amount of RuBP carboxylase protein. Thus, the altered genetic expression of RuBP carboxylase in ryegrass seems to be qualitatively and quantitatively different from that in tall fescue.

Table 1 Properties of RuBP carboxylase/oxygenase from various cultivars of ryegrass

Cultivar	Ploidy	Carboxylase			Oxygenase			Post-illumination CO ₂ burst (mg CO ₂ dm ⁻² h ⁻¹)	CO ₂ compensation point (μl l ⁻¹)
		Isoelectric point	<i>K_m</i> (CO ₂) (μM)	<i>K_i</i> (O ₂) (μM)	<i>V_{max}</i> (nmol CO ₂ per mg protein per min)	<i>K_m</i> (O ₂) (μM)	<i>K_i</i> (CO ₂) (μM)		
Gremie	2×	5.5	50	610	416	520	47	17	—
Perma	2×	5.5	49	604	425	516	48	21	—
RvP	2×	5.5	49	615	409	490	44	18	—
64038-50-308*	2×	5.5	51	620	420	485	48	20	62 ± 3
64038-1-312*	4×	5.8	22	618	432	532	30	11	52 ± 1
Barlatra	4×	5.8	22	615	416	508	25	9	—
Reveille	4×	5.8	23	605	412	525	26	12	—
Tetila Tetrone	4×	5.8	22	615	408	534	28	10	—

All assays were carried out on freshly prepared unstored enzyme. Enzyme was activated for both carboxylase and oxygenase assays by incubation for 5 min at 25 °C in 0.1 M tricine (pH 8.1) containing 20 mM MgCl₂, 10 mM NaHCO₃ and 5 mM dithiothreitol. Assays were initiated by addition of 25-μl aliquots of preincubated enzyme to assay mixtures (1 ml) containing 0.1 M tricine (pH 8.1), 5 mM dithiothreitol, 0.1 mM RuBP and with ¹⁴C₂ provided as NaH¹⁴CO₃ (0–8 mM) and O₂ (0–1,230 μM), as appropriate. RuBP was dissolved immediately before the assay to minimise the formation of xylulose biphosphate, a potent competitive-type inhibitor of RuBP binding, as described by Tolbert *et al.*⁸. All assays were carried out at 25 °C. Oxygen uptake was measured polarographically with a Clark type O₂ electrode as described by Bahr and Jensen⁹. In carboxylase assays, 50-μl samples were removed at 60 s into 150-μl aliquots of 3M HCl and the acid-stable residue was dried at 60 °C and redissolved before counting in 5-ml aliquots of the Dioxane based liquid scintillation cocktail NE 250 (Nuclear Enterprises, Edinburgh). The effective CO₂ concentrations in the assay systems were calculated using ionisation constants interpolated from the data of Harned and Bonner¹⁰. *K_i*(O₂) values were determined from Dixon plots of 1/*V* against *i* (ref. 11), the oxygen concentration. The post-illumination CO₂ burst was determined by infrared gas analysis using an open-circuit system with a quantum flux (400–700 nm) of 260 μEinstein m⁻² s⁻¹ and a temperature of 20 °C. The CO₂ compensation point was determined in a closed-circuit system at 21% O₂, 20 °C and a quantum flux (400–700 nm) of 400 μEinstein m⁻² s⁻¹.

* Isogenic lines.

Table 2 Molecular weight characteristics of isogenic diploid and tetraploid RuBP carboxylase/oxygenase isozymes

	Native enzyme	Large subunit	Small subunit
64038-50-308 (diploid)	550,000	52,000	18,000
64038-1-312 (tetraploid)	550,000	52,000	18,000

RuBP carboxylase/oxygenase was extracted and purified as described in the legend to Fig. 1. Molecular weights were estimated by column chromatography. For the native enzyme, Sepharose CL-6B-200 was used with the following molecular weight markers: thyroglobulin (670,000), catalase (250,000), γ globulin (160,000), aldolase (145,000). Subunits were prepared as described by Steer and Kernoghan¹⁶ and separated using a Sephadex G-200 column equilibrated with 0.05 M Tris, 0.001 M EDTA and 0.5% sodium dodecylsulphate, pH 8.5. The following molecular weight markers were used: bovine serum albumin (68,000), ovalbumin (45,000), pepsin (35,000), carbonic anhydrase (29,000), chymotrypsinogen A (25,000), myoglobin (17,800), haemoglobin (15,000).

The molecular basis of the alteration has not yet been established. The molecular weights of the isozymes and those of their subunits are apparently identical (Table 2). The isozymes are also indistinguishable by their antigenic properties as judged by Ouchterlony double-diffusion studies using antisera against both the diploid and tetraploid isozymes. Unfortunately, it has not been possible to produce crystalline preparations of the isozymes of sufficiently low water content to permit high resolution X-ray crystallography which might elucidate the molecular basis of the difference.

A difference in isoelectric point of 0.3 pH units (Table 1) represents a charge shift equivalent to the alteration of several amino acid residues. In CO₂, the partial charges of the oxygen and carbon atoms are -0.11 and +0.22, respectively, whereas the atoms of O₂ have no partial charge¹⁴. Thus, Ogren¹⁵ has argued that charge alterations at the active site of RuBP carboxylase are more likely to alter CO₂ binding than that of O₂, a speculation offering one possible explanation for the altered K_m (CO₂) in ryegrass. Alternatively, the charge shift inferred from the change in isoelectric point might result in a conformational change at the active site. Again, this would be expected to have a greater effect on carboxylation because the K_m of RuBP carboxylase for CO₂ is low relative to that for O₂.

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Cytochalasin B induces polarisation of plasma membrane components and actin in transformed cells

ALTERATIONS in the surface membrane associated with transition of cells from the normal to the transformed state include changes in morphology^{1,2}, membrane transport³, agglutination by plant lectins^{4,5}, contact inhibition of growth⁶ and change in the lateral mobility of membrane components⁷. It has been suggested that these changes in surface properties are related to changes in the cytoskeleton. This idea is based on the concept that microfilaments and microtubules determine the topographical distribution and the mobility of membrane components and on the observation that in several cell types microfilament bundles disappear after transformation⁸⁻¹⁰. However, there is also evidence that the presence of microfilament bundles is related to adhesiveness to the substratum and is dissociable from cellular growth control¹¹. We report here evidence for a difference in the cytoskeletal control of plasma membrane mobility between normal and transformed cells. We show that cytochalasin B (CB) induces polarisation of membrane components and actin and provokes a marked enhancement of capping in transformed but not in normal cells.

Eight normal and eight transformed cell types were exposed in suspension to CB, 10 μ g ml⁻¹ for 60-240 min after collection from their culture vessel (legend to Fig. 1, Table 1). The influence of CB on antibody-induced redistribution was studied in parallel experiments by coating cells with a single or double layer of antibodies before incubation with CB. The distribution of surface antigens and actin before and after CB treatment was monitored on fixed cells with specific antibodies, using an immunofluorescence technique. Surface antigens (H-2, β_2 -microglobulin, species specific) on all cell types not exposed to CB appeared randomly distributed (Fig. 1). The same cells, smeared and acetone-fixed, showed a diffuse staining with actin antibodies. When examined after CB treatment, none of the normal cell types, including normal 3T3 and MRC5, showed any significant alteration in morphology or in the distribution of surface antigens and actin compared with cells not exposed to CB. Also, CB did not enhance antibody-induced redistribution in the normal cell types. In contrast, in Lu106, L929, HeLa, SV40 3T3, B77 3T3 and Py6 3T3 cells, CB treatment produced a change in both morphology and the distribution of surface antigens and cytoplasmic actin (Fig. 1). In each of these cell types almost all cells formed blebs and 30-90% exhibited a single large protuberance. CB caused accumulation of actin-containing structures at the protuberances and brought membrane components to the corresponding surface area. However, the CHO cells used did not show any CB-induced rearrangements and NRK La334C14 showed relatively small perturbations.

A single layer of antibodies attached to surface antigens did not induce redistribution in any of the transformed cells examined. After crosslinking with anti-immunoglobulin the corresponding double layer generally induced patching of surface antigens in more than 50% of the cells in all cell types. The capping tendency of all cell types was weak, with caps occurring usually in only 1-3% of the cells. However, in some experiments with L cells, 15-20% of the cells exhibited caps. CB treatment of the antibody-coated cells caused polarisation of both a single and double layer of antibodies attached to the cell surface. The number of cells with such polarisation was virtually identical to that showing CB-induced redistribution of surface antigens to protuberances in the absence of antibodies (Table 1). However, the redistribution seemed most complete using a double layer of antibodies. Figure 1 shows the marked redistribution provoked by CB in transformed cells. In our

experience with conventional capping in a variety of test systems, only immunoglobulin on mouse splenocytes may exhibit a similar tendency to redistribution.

Cultivation of L cells and Lu106 cells with dibutyryl cyclic AMP for 24 h before release from the substratum and exposure to CB, altered the morphological characteristics and the detectability and organisation of actin-containing structures. The effects of dibutyryl cyclic AMP on cell shape and cytoskeletal elements have been reported elsewhere¹²⁻¹⁴. In our study, cells treated with dibutyryl cyclic AMP became either flatter or more spindle-shaped and exhibited markedly more bundles of microfilaments than untreated control cells (data not shown). The cultivation of L and Lu106 cells in the presence of dibutyryl cyclic AMP almost completely prevented the CB-induced polarisation of membrane components and the formation of protuberances. The phenotypic alterations induced by dibutyryl cyclic AMP seemed to be a requirement for inhibition of the CB effect, as a 30-min incubation of the cells in dibutyryl cyclic AMP did not interfere with the CB-induced changes.

Thus, the CB-induced polarisation of membrane components and enhancement of capping¹⁵ seem to be properties of transformed cell types which disappear on treatment with transformation-reversing agents such as dibutyryl cyclic AMP. As normal cells do not exhibit such polarisation even

after CB exposure for 240 min, this feature may reflect a profound difference between normal and many transformed cells. However, it should be noted that mouse oocytes also show a CB-induced rearrangement similar to that described here¹⁶.

There are several possible explanations for our result. Transformed cells are reported to have an impaired ability for microtubule assembly¹⁷, and microtubules may be necessary for the maintenance of the normal orientation of the microfilament system. However, the difference between normal and transformed cells is probably not due to a relative absence of microtubules, as colchicine-treated normal human fibroblasts or 3T3 did not show any polarisation of membrane antigens and actin or any protuberances. Alternatively, transformed cells may have a defective formation of some membrane component which maintains plasma membrane organisation, thus counteracting redistribution. It has been reported that after viral transformation the amount of actin associated with membranes is decreased without a parallel decrease in total cellular actin¹⁸. This selective displacement of actin from membranes may lead to a loss of structures associated with the maintenance of normal membrane organisation. Evidently, further work will be needed to elucidate the basis of the increased plasma membrane mobility of transformed cells exposed to CB.

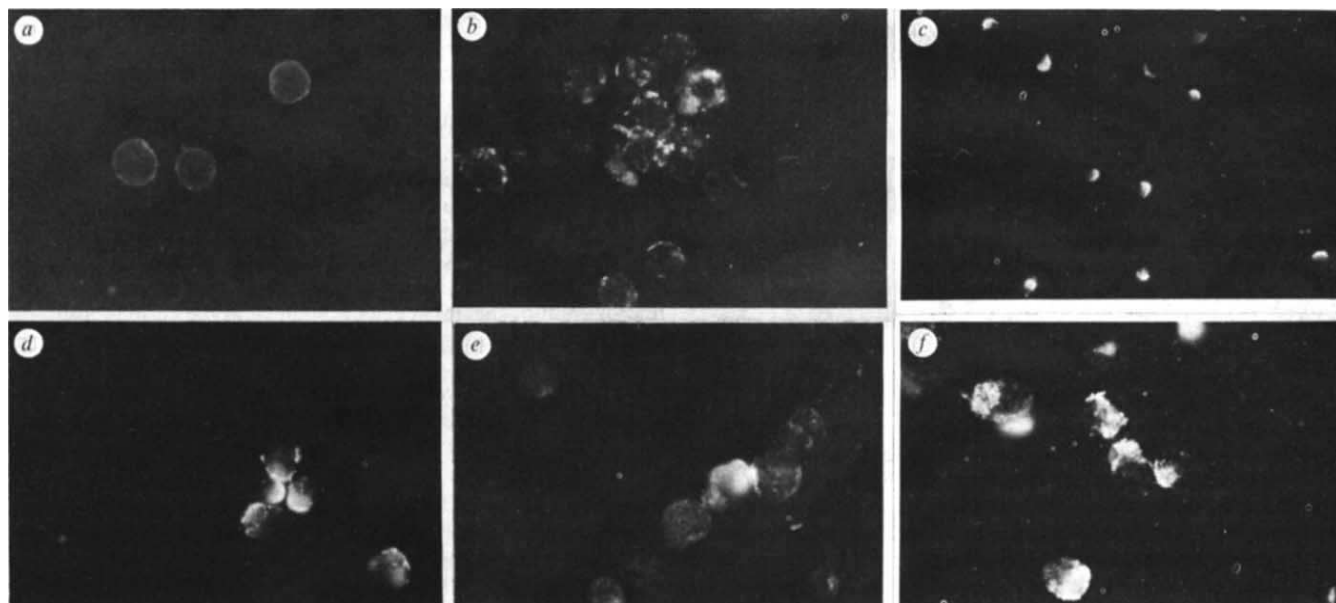


Fig. 1 Normal cells in suspension before and after exposure to CB, $10 \mu\text{g ml}^{-1}$, for 60 min at 37°C . CB was dissolved in 0.1% dimethyl sulphoxide (DMSO) and subsequently diluted in Eagle's suspension medium supplemented with 10% calf serum. CB was also included in the washing fluids. For detection of actin the cells were thereafter smeared in 34 mM sodium citrate, fixed in dry acetone at -20°C and subsequently reacted with rabbit or human actin antiserum diluted 1:15, followed by fluorescein isothiocyanate (FITC)-conjugated sheep anti-human or sheep anti-rabbit immunoglobulin (Ig), diluted 1:10. For detection of surface membrane antigens the cells were fixed in 4% paraformaldehyde (PFA) for 10 min at low temperature, whereafter the appropriate surface antigens were detected by indirect immunofluorescence staining. To study the effect of CB treatment on antibody-induced redistribution, washed cells were reacted with antibodies to surface antigens for 30 min at 4°C after release from their culture vessels, and subsequently exposed to CB. Alternatively, the cells were pretreated with antibodies to surface antigens and then with fluorescein-conjugated anti-Ig crosslinking membrane-bound antibodies. A rabbit antiserum (Dakopatts) was used for detection of β_2 -microglobulin. Species-specific antigens on mouse, rat, human and monkey cells were detected by sera prepared in rabbits. Mouse H-2 antigens were detected using either ACA anti A/Sn or C57BL anti-C3H/k antisera. Blood group A-related antigens were detected using a rabbit antiserum to purified blood group substance A. The rabbit and human antisera to actin^{19,20} and the FITC-conjugated sheep anti-human, sheep anti-rabbit and sheep anti-mouse-Ig²¹ have been described earlier. The sera were used at dilutions optimal for redistribution of cell surface antigens²². The cell types used are listed in Table 1. All cells except normal 3T3 were cultured in Eagle's minimal essential medium supplemented with a 5% calf serum. Normal 3T3 were supplemented with 10% calf serum. *a*, The distribution of H-2 antigens on SV40 3T3 after fixation with PFA (magnification $\times 490$). *b*, The same cells were reacted with antibodies and anti-Ig for 30 min each at 4°C , subsequently incubated for 60 min at 37°C , and PFA fixed. Most cells exhibit patchy fluorescence (magnification $\times 490$). *c*, The cells were reacted with antibodies as in *b* but thereafter incubated at 37°C in the presence of CB. A very marked polarisation of fluorescence is seen (magnification $\times 306$). *d*, The cells were first reacted with antibodies to surface antigens for 30 min at 4°C and thereafter incubated at 37°C for 60 min in the presence of CB before PFA fixation and subsequent detection of membrane-bound antibodies by fluorescent conjugate. In this case, a polar distribution of fluorescence is seen. Note that a 60-min incubation with a single layer of these antibodies in the absence of CB did not yield any detectable redistribution but rather a pattern similar to that in *a* (magnification $\times 490$). *e*, The reactivity of rabbit anti-actin with SV40 3T3 before exposure to CB (magnification $\times 490$). *f*, The reactivity of the same antiserum after CB treatment (magnification $\times 490$). The intensity difference between *e* and *f* reflects the visual impression obtained in the microscope.

Table 1 Effects of cytochalasin B on normal and transformed cells in suspension

Cell type	Characteristics	Morphology	Distribution of surface membrane components*	Distribution of cytoplasmic actin†
Human skin fibroblasts 3–4th passage	Normal	Unaffected	Unaffected (β_2 -microglobulin (β_2 -m); species specific)	Unaffected
Rat lung fibroblasts (primary culture)	Normal	Unaffected	Unaffected (species specific)	Unaffected
Mouse embryo fibroblasts (A/Sn) (2–4th passage)	Normal	Unaffected	Unaffected (H-2; species specific)	Unaffected
Mouse kidney cells 1st passage (A/Sn)	Normal	Some blebs	Unaffected (H-2; species specific)	Unaffected
Monkey kidney cells primary culture, 1st passage	Normal	Unaffected	Unaffected (species specific; blood group A)	Unaffected
MRC 5 (human line)	Normal	Unaffected	Unaffected (species specific; β_2 -m)	Unaffected
Mouse 3T3	Normal	Unaffected	Unaffected (H-2; species specific)	Unaffected
NRK (rat kidney cells)	Normal	Unaffected	Unaffected (species specific)	Unaffected
Lu106	Transformed	Blebs 20–70% protuberances	10–50% polar (β_2 -m; species specific)	Polar
L929	Transformed	Blebs 60–90% protuberances	50–80% polar (H-2; species specific)	Polar
Hela	Transformed	20–40% protuberances	10–30% polar (β_2 -m; species specific)	Polar
CHO	Transformed	Blebs	Unaffected (species specific)	Unaffected
B77 3T3‡	Transformed	Blebs 30–60% protuberances	30–50% polar (H-2; species specific)	Polar
SV40 3T3	Transformed	40–70% protuberances	30–60% polar (H-2; species specific)	Polar
Py6 3T3§	Transformed	30–50% protuberances	20–40% polar (H-2; species specific)	Polar
NRK La334C14	Transformed	Blebs 5% protuberances	1–5% polar (species specific)	Unaffected/polar

* The % given are based on 3–14 separate experiments and represent maximum and minimum values obtained in these experiments. The figures also apply to cells coated with a single or a double layer of antibodies to surface antigens before treatment with cytochalasin B.

† The frequency of cells showing polarisation of actin corresponds to the number of cells with altered morphology.

‡ Transformed with Bratislava 77 RNA tumour virus.

§ Transformed with polyoma virus.

|| Transformed with Rous sarcoma virus.

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Immunoglobulin μ and γ heavy chain classes are not determined by class-specific RNA-splicing enzymes

An immunoglobulin (Ig)-producing lymphocyte can sometimes express one heavy-chain variable (V_H) region combined with different heavy-chain constant (C_H) regions. For example, single cells making IgM and IgD with the same antibody specificity have been observed¹. Cells, both singly² and in mass culture^{3,4}, switch from IgM to IgG production, and it is believed that the variable regions expressed are the same before and after the switch. We describe here the analysis of a hybrid derived by fusing an IgM-producing cell with an IgG producer, where the corresponding μ and γ heavy chains bear different variable regions, denoted V_1 and V_2 . This hybrid makes only the parental heavy chains— $V_1C\mu$ and $V_2C\gamma$ —and not the novel combination $V_2C\mu$. This result suggests that the μ and γ heavy-chain classes are not determined by class-specific RNA processing.

Two models have been proposed to account for the expression in one cell of the same V_H region combined with more than one C_H region. On the one hand, the appropriate DNA sequences might be translocated so that in a cell making IgM the V_H region would be adjacent to the μ -constant ($C\mu$) region, thus providing a template for μ -encoding RNA. Subsequent translocation which brought V_H and $C\gamma$ together would permit transcription of an RNA for the complete γ chain, and cause the cell to switch from IgM to IgG production.

The phenomenon "RNA splicing" suggested an alternative model for the activation of heavy chain genes^{5,6}. First, translocation would bring together the cluster of C_H ($C\mu C\delta C\gamma$) and a particular V_H gene. An RNA for the entire $V_H C\mu C\delta C\gamma$ region would be transcribed. For an IgM producer, this primary

transcript would be processed so that the V_H region would be in tandem with $C\mu$, whereas for an IgG producer, the RNA would be processed so that V_H and $C\gamma$ lay in tandem, thus offering an elegant mechanism for the heavy-chain class switch.

The RNA-processing model requires that a hybrid cell coexpressing μ ($V_1C\mu$) and γ ($V_2C\gamma$) have both μ - and γ -processing enzymes, and that these enzymes act on the primary transcripts of both the V_1 - and V_2 -expressing chromosomes, thus predicting that the hybrid should make the scrambled chains $V_1C\gamma$ and $V_2C\mu$ as well as the parental $V_1C\mu$ and $V_2C\gamma$ chains.

We have tested this hypothesis using the hybridoma cell line Sp2/HLML'. This hybridoma was derived from the fusion of Sp2/HL-Ag14 (ref. 7), which makes an IgG2b specific for sheep red cells (SRC), with a syngeneic spleen cell making an IgM of unknown specificity. As a test for the presence of scrambled molecules, we have looked for IgM with SRC specificity, and also for direct SRC plaques.

Hybridoma cells occasionally yield progeny which no longer express one or more of the light or heavy chains. Therefore we used a recent Sp2/HLML' reclone in these experiments. Twelve further reclones of this reclone were tested and each was found to synthesise the μ and γ heavy chains and both light (L and L') chains.

To ascertain whether the light chain involved in SRC specificity combines with the μ chain, we separated the IgG2b from the IgM by their affinity for protein A and concanavalin A, respectively (see legend to Fig. 1). The γ 2b chain associates preferentially with the light chain donated by the IgG2b parent, whereas the μ chain associated equally well with both light chains (Fig. 1). By counting the radioactivity in the μ and γ bands, we found that this hybrid cell line makes about 1.4

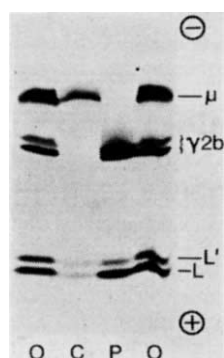


Fig. 1 The hybridoma cell line Sp2/HLML' was derived by fusing a spleen cell making IgM of unknown specificity with the SRC-specific IgG2b secreting hybridoma Sp2/HL-Ag14. Sp2/HL-Ag14 is itself an azaguanine-resistant mutant of Sp2/HL which was a reclone of Sp2/HLGK (ref. 7) that no longer makes the nonspecific X63-Ag8 γ , and κ chains. Ig radioactively labelled with ^{14}C -leucine was prepared as described previously¹². To prepare separate IgG2b and IgM fractions, we took advantage of conditions in which protein A binds to IgG2b but not IgM (ref. 13), and that concanavalin A (Con A) binds IgM (but not IgG2b or free light chains)¹⁴. A sample (10 ml) of cell culture supernatant was passed over a 0.7 ml protein A Sepharose (Pharmacia) column and 6 ml of the eluate was then passed over a 1 ml Con A Sepharose (Pharmacia) column. The Con A column was then washed with several volumes of PBS, and the IgM was removed by passing several volumes of 1 M α -methylmannoside in 50% PBS. The protein A column was also washed with PBS and then the IgG2b was eluted by passing several volumes of 0.1 M acetic acid in 0.9% NaCl over the column. The protein A binding, acid-eluted fractions (P) and the Con A binding, mannose-eluted fractions (C) were dialysed against PBS and their Ig content was assayed by measuring the endpoint dilution of protein A lysis¹⁵ or of haemagglutination (Table 1 legend). The fractions containing the highest Ig concentration were analysed by polyacrylamide gel electrophoresis (PAGE) as described previously¹². The light chains L and L' were identified by comparing their mobility with the L chain secreted by Sp2/HL-Ag14 (data not shown).

times as much μ as γ . Therefore, if the SRC-specific V_H associated with the $C\mu$, we would expect to detect SRC-specific IgM.

We measured SRC binding by assaying haemagglutination which was rendered more sensitive by including class-specific enhancing serum. We do not have preparations in which IgG and IgM bear the same V region, and therefore we could not measure accurately the relative sensitivity of the IgG and IgM haemagglutination assays. As an alternative, we compared the SRC-specific IgG2b with the SRC-specific IgM made by two different hybridomas, Sp1/HLGK (ref. 7) and Sp25/5-1 (ref. 8), both of which make the IgG1 of X63-Ag8 in addition to the SRC-specific IgM. Using ^{14}C -leucine labelled supernatants of these cell lines, we have measured the μ and γ content and the haemagglutination titres and found that our IgG2b and IgM haemagglutination assays could detect SRC-specific IgG2b and IgM, respectively, equally well (Table 1).

Table 1 presents the haemagglutination tests for SRC-specific IgM. To monitor IgM recovery, we added to the Sp2/HLML' supernatant a relatively small amount of supernatant of the Sp6 cell line which makes IgM specific for the hapten, trinitrophenyl (TNP) γ . IgM concentration was followed by lysis of TNP horse red cells, as well as by lysis of protein A red cells. The cell culture supernatant of Sp2/HLML' was mixed with protein A Sepharose, which removed more than 95% of the IgG2b. This step was necessary both to reduce the background IgG2b SRC activity, and to eliminate the possibility that an excess of the SRC-specific IgG2b would block the binding of a small amount of SRC-specific IgM. The IgM in the material not binding to protein A was then concentrated by ammonium sulphate precipitation, resuspended and dialysed. This fraction showed no IgM SRC haemagglutination activity, indicating that less than 0.15% of the total IgM was SRC specific.

Cells secreting SRC-specific IgM make a direct plaque, that is, they require only complement to lyse red cells. Cells secreting SRC-specific IgG2b make only indirect plaques—they require a developing antiserum to make a plaque after a short incubation. As a test for rare Sp2/HLML' cells making substantial amounts of SRC-specific IgM, a total of 4.5×10^7 Sp2/HLML' cells were plated: either 8×10^6 , 4×10^6 , 2×10^6 , or 1×10^6 cells were added to each of three plates. Included in one plating at each dilution were 10^3 Sp1 cells, which secrete SRC-specific IgM. In these conditions the plaquing efficiency of Sp1 ranged from 16 to 22%. No plaques were observed, however, when Sp2/HLML' cells were plated alone. We conclude that the frequency of Sp2/HLML' variants secreting SRC-specific IgM is <1 in 5×10^6 (1 in 3×10^7 multiplied by 16%).

In a previous study, no indirect plaque-forming cells could be demonstrated among 4×10^5 cells of the hybridoma line Sp1/HLGK which secretes SRC-specific IgM and IgG1 of unknown specificity⁹. A weakness of this previous experiment was that whereas the SRC-specific variable region presented on pentameric IgM sufficed to give plaques, this same variable region on monomeric IgG1 might have been of too low avidity to cause plaques. Here we have avoided this weakness by analysing the reverse situation.

The existence of high (20%) levels of scrambled molecules has been excluded by isoelectric focusing analysis in the case of a hybrid cell line secreting γ 1 and γ 2a molecules¹⁰. Here we have shown that in a hybrid cell making μ and γ molecules, less than 0.15% of the μ molecules bear the SRC-specific variable region donated by the γ 2b synthesising parent. The stability of parental V-C expression argues against the hypothesis that expression of heavy chains of different classes is determined by class-specific RNA splicing, and therefore suggests that class expression is determined by a condition that exerts its effect before RNA transcription, such as the translocation of the DNA encoding the V_H and C_H in question.

Although we have shown that regulation of the IgM to IgG switch is probably not due to a change in RNA splicing of the

Table 1 Haemagglutination tests for SRC-specific IgM

Preparation tested	Reciprocal haemagglutination titre		Reciprocal TNP-HRC lysis titre	SRC-specific IgM/Total IgM	
	No enhancing serum	Enhancing serum for			
		IgM	IgG		
Crude supernatant of Sp2/HLML' + Sp6	No aggl.	3 ¹	3 ⁵	3 ³	< 1.3%
Protein A non-binding unconcentrated	No aggl.	No aggl.	3 ⁰	3 ³	< 0.4%
Concentrated	No aggl.	No aggl.	3 ¹	3 ⁴	< 0.15%
Crude supernatant of Sp2/HL-Ag14	No aggl.	3 ²	3 ⁶	No lysis	—
Amount protein (c.p.m. per PAGE band)					
	in μ	γ			
Sp2/HLML	5,940 (100%)	4,560 (76%)	No aggl.	No aggl.	3 ³
Sp1/HLGK	2,260 (38%)		No aggl.	3 ²	3 ⁰
Sp25/5-1	1,120 (19%)		No aggl.	3 ²	3 ⁰

Culture supernatants were obtained after growing cells to high density. To the Sp2/HLML' supernatant was added a relatively small amount of supernatant of the cell line Sp6 which contained IgM specific for the hapten trinitrophenyl (TNP)⁷, so that IgM recovery could be monitored by the lysis of TNP horse red cells (HRC). Protein A binds IgG2b but not IgM (ref. 13), providing a simple method of removing the IgG2b from the culture supernatant. 10 ml of the Sp2/HLML' with 1 ml of the Sp6 supernatants were mixed together with 1 ml protein A Sepharose (Pharmacia), agitated for 20 min, and centrifuged. Ig was concentrated by precipitation with ammonium sulphate: 1.95 g ammonium sulphate was added to 8 ml of the protein A Sepharose supernatant. This suspension was incubated overnight at 4 °C and then centrifuged for 5 min at 2,500g. The pellet was resuspended in 0.1 ml PBS and dialysed extensively against PBS. The amount of SRC-specific Ig was assayed by measuring the haemagglutination dilution endpoint in the presence of the appropriate enhancing serum. To enhance the haemagglutination by the IgG2b, we used an antiserum (final dilution 1:100) from a rabbit immunised with the IgG1 protein of MOPC 21 (ref. 16). This antiserum is somewhat nonspecific, reacting with IgG of several subclasses, as well as the κ light chain, but enhances IgM agglutination only poorly. To enhance haemagglutination by IgM we used a μ -specific antiserum (final dilution 1:100) that had been prepared by immunising a sheep with purified MOPC 104E μ chains. The serum was absorbed on a MOPC 21 (IgG1)-Sepharose column. Total Ig was assayed by measuring protein A lysis dilution endpoint¹⁵. IgM was monitored by measuring TNP HRC lysis, on the assumption that Sp6 IgM and Sp2/HLML' IgM would be affected similarly by the preparative steps used here. To calculate the limit of the fraction of IgM that may have SRC activity, we assumed that the sensitivity of detecting SRC-specific IgM is the same as for IgG (see text). To ascertain the amount of μ and γ made by the Sp2/HLML', Sp1/HLGK, and Sp25/5-1 hybridomas, the corresponding bands from an SDS PAGE gel were cut out, dissolved by incubating them in 30% H₂O₂ at 60 °C for 20 h, and counted for radioactivity.

same transcript, the RNA splicing model might be valid for the regulation of the expression of other classes. As already mentioned, the membrane of some normal B cells contains both μ and δ bearing the same V region¹. Hybridomas derived from the fusion of transformed hamster cells with a mouse spleen cell making both mouse μ and δ have been isolated¹¹, suggesting that cells synthesise these two heavy chains simultaneously. As described here for the fusion of IgG- and IgM-producing cells, the fusion of the μ - δ doubly producing cells with cells making an IgM with a distinct specificity, might provide the material to ascertain how the μ and δ classes are determined.

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***In vitro* synthesis of a functional avian sarcoma virus transforming-gene product**

A SINGLE avian sarcoma virus (ASV) gene (*src*) is responsible for the induction and maintenance of cell transformation *in vitro* and tumour production in infected animals¹. The product of the ASV *src* gene is a phosphoprotein with an apparent molecular weight of 60,000 and has been designated p60^{src} (refs 2-4). This *src* protein seems to act as a protein kinase when assayed by the transfer of ³²P from γ -labelled ATP to IgG in specific immune complexes⁵. As this protein kinase activity is growth and temperature dependent in chick cells transformed with an ASV temperature-sensitive transformation mutant⁵, these results suggest that ASV may transform cells by aberrant phosphorylation of cellular proteins. Radiolabelled p60^{src} is synthesised in cell-free extracts programmed by the 3' third of the viral RNA, the region of the genome which contains the *src* gene, but not by similar RNA from a mutant which has a deletion of the *src* gene^{3,4}. Because of the structural similarity between the *in vitro* and *in vivo* products⁴, we tested the *in vitro* translation product for phosphotransferase activity. We have found that it exhibits protein kinase activity, providing direct evidence that p60^{src} is the functional product of the avian sarcoma virus transformation gene.

The viruses used in this study were Schmidt-Ruppin strain, subgroup D, and a transformation defective (td) strain of Schmidt-Ruppin, subgroup D. RNA was extracted from nondefective (nd) and from td purified virions. The 70S RNA was heat-denatured and the poly A-containing molecules were purified by oligo-dT cellulose chromatography as previously described⁴. The poly A-containing RNA was fractionated by

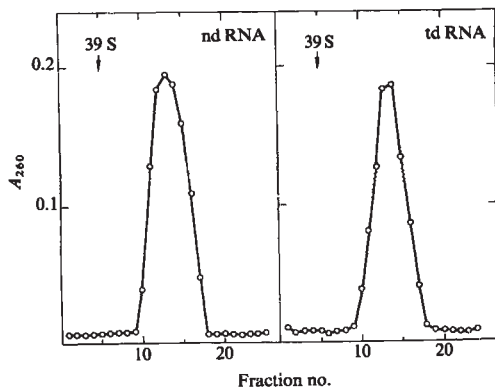


Fig. 1 Sedimentation profile of nd and td poly A-containing RNA selected for translation. RNA selected from a previous sucrose gradient was precipitated with ethanol, dissolved in EDTA 1 mM, Tris-HCl 0.01 M (pH 7.2), heated at 80 °C for 2 min, quickly chilled and sedimented for 105 min through a 20 to 5% (w/v) sucrose gradient containing 0.1 M NaCl at 10 °C and 45,000 r.p.m. in a Beckman SW50.1 rotor. The RNA in fraction nos 12–15 was ethanol-precipitated and used for cell-free translation as described in the legend to Fig. 2.

sucrose gradient sedimentation and RNA of approximately one-third genome length was selected. This RNA was resedimented (Fig. 1) and the RNA in fraction numbers 12–15 was used to programme messenger-RNA-dependent reticulocyte lysates as described in the legend to Fig. 2.

The ^{35}S -methionine-labelled polypeptide products analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis show that p60^{src} is translated only from nd RNA (Fig. 2, tracks 1–3). No p60^{src} is translated from td RNA although this RNA stimulated protein synthesis ninefold above the background synthesis without RNA, compared with the 10-fold stimulation with nd RNA. We have no information concerning the nature of the polypeptides synthesised in response to td RNA except that one migrates with Pr76, the product of the ASV *gag* gene, indicating that even this highly selected subgenomic RNA still contains some sequences from the 5' end of the genome. In contrast, the major translation product of this size class of nd RNA is p60^{src} as previously described^{3,4}.

Serum from rabbits bearing ASV-induced tumours (TBR serum) was used to immunoprecipitate the *in vitro* translation products by the same procedures used to precipitate p60^{src} -associated protein kinase from transformed cell extracts⁵. This serum is capable of precipitating both virion structural proteins and the product of the *src* gene, p60^{src} (ref. 2). The radiolabelled polypeptides found in the immunoprecipitates are shown in Fig. 2, tracks 4–7. Immune serum from TBR precipitates p60^{src} and a small amount of Pr76 from the products synthesised in response to nd RNA (track 5) and, in addition, a small amount of Pr76 is found in the immunoprecipitate of the products synthesised in response to td RNA (track 7). This shows that radiolabelled virus-specific polypeptides synthesised in cell-free extracts are antigenically similar to those found in transformed cells².

A portion of the immune complexes formed with the translation products shown in Fig. 2 was also resuspended directly in the kinase reaction buffer, and the transfer of γ -labelled phosphate from ATP to the heavy chain of IgG was detected by SDS-polyacrylamide gel electrophoresis of the reaction products. Figure 3 shows that only the products of translation of nd RNA immunoprecipitated with immune serum (TBR) yielded phosphorylated IgG. Quantitation of this phosphorylated polypeptide (Fig. 3, track 4) revealed that translation of 1.5 pmol of nd RNA resulted in sufficient protein kinase activity to transfer 3.2 fmol of ^{32}P to IgG when immune serum was used for precipitation, whereas no activity was observed when normal

rabbit serum was used. Translation of the same amount of td RNA resulted in no detectable enzymatic activity with immune serum (Fig. 3, track 3). These results are consistent with those previously observed with immunoprecipitates from infected cell lysates where only those immune complexes which contained p60^{src} showed phosphotransferase activity⁵.

The translation of viral RNA *in vitro* resulting in the synthesis of an enzymatically functional p60^{src} strongly indicates that the product of the *src* gene is a protein kinase. However, it is possible that the observed enzymatic activity results from a specific association between a cellular kinase and p60^{src} . This seems unlikely as such a cellular kinase would have to be present in both avian and mammalian cells as well as in the reticulocyte cell-free extracts used here.

Several reports have indicated that the ASV *src* gene is translated in cell-free extracts into a variety of polypeptides smaller than 60,000 daltons^{10–12}. To date, we have been unable to detect these smaller proteins in transformed cells, although it is possible that the antiserum from tumour-bearing rabbits does not recognise transformation-specific polypeptides other than p60^{src} . Regardless of whether these polypeptides are present in transformed cells, the results we present here and elsewhere indicate clearly that p60^{src} is a product of the ASV transformation gene both *in vitro* and *in vivo* and that its function is likely to result in the phosphorylation of cellular proteins. In view of the well documented role of protein phosphorylation in the regulation of cellular processes, this activity alone may be

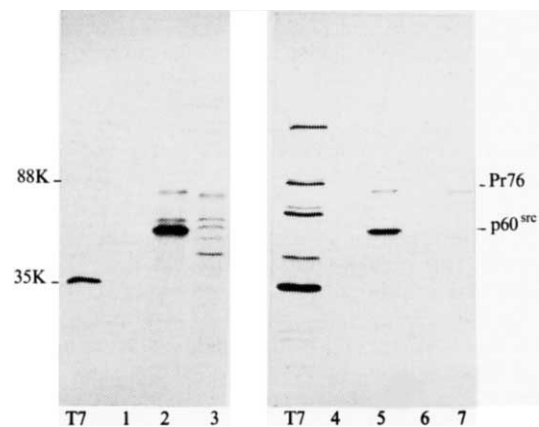


Fig. 2 Fluorogram of SDS-polyacrylamide gel electrophoresis analysis of ^{35}S -methionine-labelled polypeptides synthesised in cell-free extracts programmed by the RNA shown in Fig. 1. Messenger-dependent reticulocyte lysates were prepared as described by Pelham and Jackson⁶ and the conditions of translation were as described elsewhere³ except that the final concentrations were 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM Mg acetate, 100 μM unlabelled methionine and 0.5 μCi μl^{-1} ^{35}S -methionine (700 Ci mmol^{-1} , NEN). 3 pmol of nd RNA was added to each of two 300- μl reaction mixtures, 3 pmol of td RNA was added to one 300- μl reaction mixture, and the reaction mixtures were incubated at 30 °C for 40 min. Samples were then taken for the determination of incorporation, for polyacrylamide gel analysis, and one-half of each reaction was used for determination of enzymatic activity. Stimulation of 0.3 M KOH-resistant (15 min at 37 °C) and 10% trichloroacetic acid-precipitable radioactivity was 10-fold above background for the lysate programmed with nd RNA and ninefold for that programmed with td RNA. The direct analysis of these products is shown: track 1, no RNA; track 2, nd RNA; track 3, td RNA. Immunoprecipitation of the translation products was carried out with normal rabbit serum or serum from tumour-bearing rabbits (TBR) and the immunoprecipitated polypeptides are shown: track 4, nd RNA, normal serum; track 5, nd RNA, TBR serum; track 6, td RNA, normal serum; track 7, td RNA, TBR serum. Immunoprecipitations were carried out as previously described² using the procedure of Kessler⁷. Before electrophoresis, samples were boiled in sample buffer (0.07 M Tris-HCl (pH 6.8), 11.2% glycerol, 3% SDS, 0.002% bromophenol blue, 5% β -mercaptoethanol). Electrophoresis⁸ and fluorography⁹ were carried out as previously described⁴. T7 virion proteins are included as molecular weight markers.

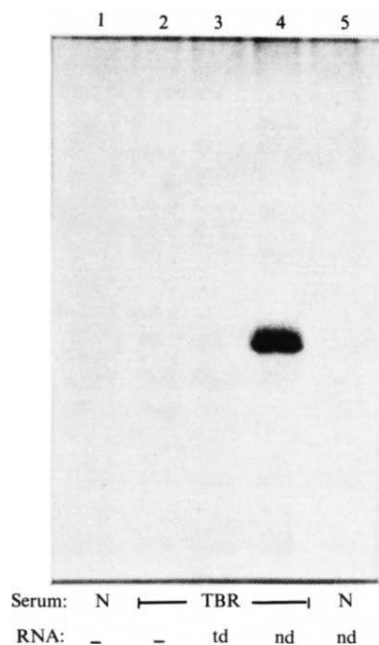


Fig. 3 Autoradiogram of SDS-polyacrylamide gel analysis of phosphorylated peptides. A portion of the bacteria-bound immune complexes obtained after protein synthesis and immunoprecipitation was resuspended directly in 25 μ l of reaction buffer containing 20 mM Tris-HCl (pH 7.2), 5 mM $MgCl_2$ and 1 μ M [γ - ^{32}P] ATP (1,000 Ci $mmol^{-1}$) as previously described⁵. After 10 min at 30 °C the bacteria-bound immune complexes and any phosphorylated polypeptides were washed and then resuspended in sample buffer and boiled for 1 min to release any polypeptides bound to the bacteria. After electrophoresis the gel was fixed in 10% acetic acid-5% methanol and washed overnight. The phosphorylated polypeptides were detected by exposure of the dried gel for 1 h to Kodak-X-Omat film at -70 °C using a DuPont Cronex Hi-Plus intensifying screen to enhance detection.

sufficient to initiate and maintain neoplastic transformation. Identification of the target(s) of p60^{src} phosphorylation and the further characterisation of the ASV *src* gene product should permit resolution of the apparent discrepancies.

The Schmidt-Ruppin strain, subgroup D, was originally obtained from John Wyke, and the td strain from Peter Vogt. This research was supported by grants CA-2117, CA-15823 and CA-21326 from NIH, and grant VC-243 from the American Cancer Society. M.S.C. is supported by grant DRG-181-F from the Damon Runyon-Walter Winchell Cancer Fund.

Note added in proof: The enzymatic activity observed here cannot be ascribed to the translation of virus-specific mRNA from cells sequestered by virions because identical results were obtained when these experiments were repeated with the 3' third of the viral RNA selected, as described previously⁴, from T1 RNase-digested full-length (39 S) genome RNA.

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Evidence for translation of rabbit globin mRNA after liposome-mediated insertion into a human cell line

THE ability to insert messenger RNA selectively into large numbers of differentiated eukaryotic cells and thereby alter the expression of the eukaryotic genome would provide a new and direct approach to the study of the molecular mechanisms involved in protein synthesis. Several procedures have been used to facilitate the cellular incorporation of administered RNA, including microinjection of RNA into *Xenopus* oocytes¹⁻⁶ and into fully differentiated eukaryotic cells^{7,8}, direct co-cultivation of cells with naked RNA (refs 9-12), and encapsulation of mRNA within erythrocyte ghosts followed by Sendai virus induced fusion of the ghosts to recipient cells in culture¹³. We describe here the use of liposomes for the selective insertion of functional mRNA into differentiated eukaryotic cells *in vitro*. We provide evidence which indicates that cultured human epithelial carcinoma cells (HEp-2) treated with liposomally encapsulated rabbit globin mRNA are stimulated to produce a globin-like protein.

Globin mRNA was isolated from the reticulocytes of rabbits suffering from phenylhydrazine-induced anaemia¹⁴ by the method of Aviv and Leder¹⁵ and sequestered within liposomes¹⁶ by the technique of Ostro *et al.*¹⁷ The liposomes were mixed with HEp-2 cells in the presence of 3H -amino acids either with or without transcription inhibitors (actinomycin D or α -amanitin). The cells were lysed and the soluble proteins purified by ammonium sulphate precipitation followed by passage through an immunoadsorbent column consisting of Sepharose 4B covalently linked to the IgG fraction of goat anti-rabbit globin antiserum¹⁸.

The proteins eluted from the immunoadsorbent columns were analysed by electrophoresis on SDS polyacrylamide gels. The distribution of radioactivity of the proteins isolated from cells which had been incubated with liposomally sequestered globin mRNA was compared to that of the proteins derived from untreated cells (Fig. 1a). All liposome-treated preparations had three major peaks of radioactivity which occurred in fractions 15, 20 and 24 with R_f values of 0.38, 0.50 and 0.75, respectively. The latter value corresponded to the R_f of the rabbit globin standard. Although the first two peaks (R_f 0.38, 0.50) occurred in proteins isolated from non-liposome-treated control cells, a peak co-electrophoresing with the globin standard (R_f 0.75) was completely absent (Fig. 1a). When HEp-2 cells treated with liposomally sequestered globin mRNA were grown in the presence of actinomycin D, the peak of radioactivity corresponding to the globin standard was enhanced whereas the two main contaminating protein peaks were eliminated (Fig. 1b). Also, peptides migrating further than the globin subunit were noted which were obvious only in the lysates obtained from cells treated with liposome-encapsulated mRNA and grown in the presence of actinomycin D. These smaller peptides may possibly represent either incomplete translation products or partially degraded globin. When HEp-2 cells treated with empty liposomes and exogenously added globin mRNA were grown in the absence of actinomycin D, the two major protein contaminants were present but no peak of radioactivity co-migrated with the globin standard (Fig. 1b). Additional controls consisted of protein isolates obtained from cells grown in the presence of actinomycin D which were either liposome-untreated or incubated with empty liposomes and exogenously added globin mRNA. In both instances (data not shown), all the major peaks of radioactivity were absent.

Proteins obtained from HEp-2 cells grown in the presence of α -amanitin and treated with either liposomally sequestered globin mRNA, or empty liposomes and exogenously added mRNA, were analysed on 2-20% sucrose gradients alone, in the presence of monospecific goat anti-rabbit globin antibody, or combined with antibody and excess nonradioactive globin

(Fig. 2). The sucrose gradient profiles show that although other proteins appear, isolates obtained from cells treated with liposomally sequestered mRNA exhibit a peak of radioactivity co-sedimenting with the globin subunit standard. This peak is

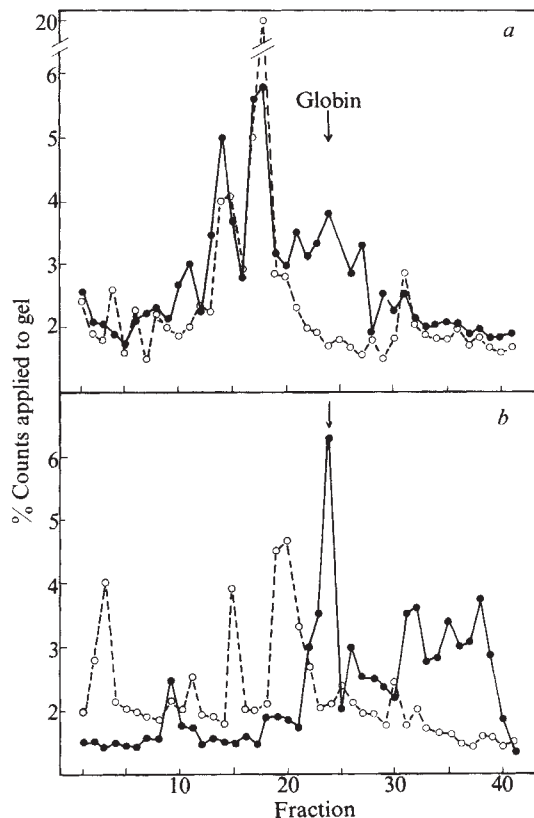


Fig. 1 SDS polyacrylamide gel electrophoresis of a mixture of radioactive HEP-2 cell protein isolates (previously purified by immunoabsorbent chromatography) and rabbit globin standard. Ether infusion liposomes, consisting of lecithin and dicetylphosphate in an 8:2 molar ratio and containing rabbit globin mRNA, were prepared by the method of Deamer and Bangham¹⁶ as adapted by Ostro *et al.*¹⁷ for the sequestration of RNA. HEP-2 cells were mixed with 1 ml of liposomes on a tumbledrum apparatus for 1 h at 37 °C in the presence of 250 µCi of ³H-amino acids and 5 ml of serum-supplemented minimal essential medium. Subsequently, the liposome-cell mixture was added to 30 ml of RPMI-1640 medium containing 100 µCi of ³H-amino acids and incubated at 37 °C overnight. To inhibit endogenous protein synthesis, actinomycin D (5 µg ml⁻¹) or α -amanitin (2 µg ml⁻¹) was mixed with the HEP-2 cells either 3 h (actinomycin D) or 6 h (α -amanitin) before the addition of liposomes. These levels of the transcription inhibitors were maintained during the 1 h of liposome-cell co-cultivation as well as during a subsequent 5 h incubation period. The cells were then lysed and the soluble proteins isolated as described in the text. Lyophilised protein samples obtained from cells treated with liposomally sequestered globin mRNA were analysed on 10% SDS polyacrylamide gels by the method of Weber and Osborn²⁴. Samples containing between 5×10^3 and 2×10^4 counts were applied to the SDS gels in the presence of 100 µg of rabbit globin standard and electrophoresed for 5 h. The gels were then fixed in methanol, water, acetic acid (227:227:46) overnight and stained in 12.5% trichloroacetic acid (TCA) containing 0.1% Coomassie blue. After the gels had been analysed for the presence of protein bands by scanning at 600 nm, they were cut into 2-mm slices, solubilised at 60 °C in a 2:1 mixture of 30% hydrogen peroxide and 60% perchloric acid, and counted. *a*, ●, Radioactivity profile of proteins from HEP-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from untreated HEP-2 cells. *b*, ●, Radioactivity profile of proteins from HEP-2 cells grown in the presence of 5 µg ml⁻¹ actinomycin D and treated with liposome-sequestered globin mRNA; ○, radioactivity profile of HEP-2 cells treated with empty liposomes and 100 µg of exogenously added globin mRNA. Peaks at fractions 15, 20 and 24 have R_f values of 0.38, 0.50 and 0.75, respectively.

absent in control cell lysates (Fig. 2a). When purified monospecific antibody was mixed with the experimental cell protein isolates, the peak of radioactivity corresponding to the globin subunit standard was shifted to the bottom of the gradient

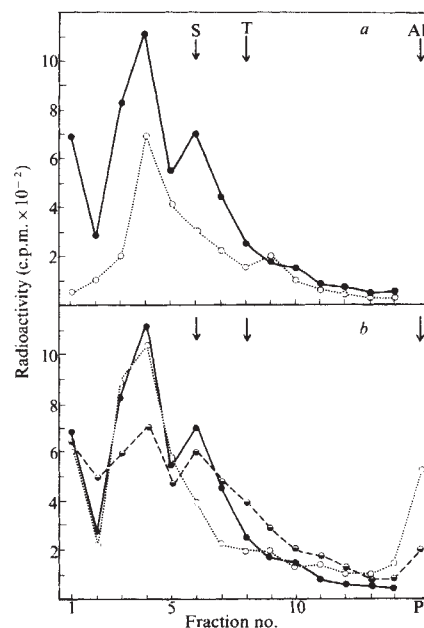


Fig. 2 Sedimentation analysis on 2–20% sucrose gradients of HEP-2 cell protein isolates (previously purified by immunoabsorbent chromatography) in the presence of monospecific goat anti-rabbit globin antibody. A goat was immunised with pure rabbit globin in complete Freund's adjuvant. The antiserum obtained gave a single precipitin arc with rabbit globin by immunoelectrophoresis. To remove non-antibody proteins, the antiserum was passed through an immunoabsorbent column consisting of Sepharose 4B covalently linked to rabbit globin¹⁸. The purified antibody was eluted with 0.2 M glycine sulphate, pH 2.3, and concentrated by ultrafiltration. The resulting antibody preparation also gave a single arc with rabbit globin by immunoelectrophoresis and did not react with a concentrated protein isolate (30 mg of protein per ml) from untreated HEP-2 cells. Protein isolates obtained from experiments using α -amanitin (2 µg ml⁻¹) were analysed on 2–20% sucrose gradients in the presence of monospecific goat anti-rabbit globin antibody. Gradients were prepared in 0.05 M Tris buffer, pH 7.5, 0.05 M KCl, 5 mM MgCl₂ and were centrifuged in an SW41 rotor at 40,000 r.p.m. for 30 h. The following samples were analysed: protein derived from cells treated with liposomally sequestered mRNA (experimental lysate); protein derived from cells treated with empty liposomes and exogenously added mRNA (control lysate); separate experimental and control lysates plus 1 mg of monospecific goat anti-rabbit globin antibody; separate experimental and control lysates and antibody plus 1.5 mg of pure globin; globin subunit, globin tetramer and antibody standards. All gradients were fractionated into 0.6-ml aliquots on an Isco density gradient fractionator. Fractions derived from gradients containing globin and antibody standards were monitored at 280 nm, and fractions from gradients containing radioactive protein isolates were TCA precipitated onto Millipore filters and counted. *a*, ●, Radioactivity profile of proteins from HEP-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from HEP-2 cells treated with empty liposomes and exogenously added globin mRNA. *b*, ●, Radioactivity profile of proteins from HEP-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from HEP-2 cells treated with liposome-sequestered globin mRNA and mixed sequentially with excess nonradioactive globin and antibody before centrifugation. S, subunit standard; T, tetramer standard; Ab, antibody marker; P, pellet.

coincident with the antibody marker. The addition of excess nonradioactive globin to the experimental cell lysate before the addition of antibody inhibited this shift (Fig. 2b).

We have demonstrated by SDS gel electrophoresis and sucrose density gradient analysis that HEP-2 cells, treated with liposomally encapsulated globin mRNA, are stimulated to synthesise a previously nonexistent globin-like protein product which reacts with monospecific goat anti-rabbit globin antibody. Sucrose density gradient analysis of the HEP-2 cell translation products revealed the presence of a globin subunit-like protein molecule. However, the assembled globin tetramer ($\alpha_2\beta_2$) is not seen, perhaps because of the lack of haemin in the recipient HEP-2 cell or the selective binding of β -chain message to specific protein synthesis initiation factors¹⁹. Whether these or other factors affect globin assembly in our system remains to be determined. Although the data presented strongly indicate that globin subunits are produced by HEP-2 cells treated with liposomally sequestered globin mRNA, further chemical analysis of the purified product, such as peptide mapping and isoelectric focusing, must be carried out to prove unequivocally that the protein product is in fact globin.

Multilamellar liposomes (500 Å diameter) and small unilamellar liposomes (200 Å diameter) have been used previously to entrap and deliver into cells a wide variety of macromolecules²⁰⁻²³. However, until recently the ability of liposomes to sequester high molecular weight RNA had not been demonstrated¹⁷, possibly due to the limited trapping efficiency of the standard small liposomes. By using large unilamellar liposomes produced by the ether infusion technique of Deamer and Bangham¹⁶, steric problems can be overcome and large molecules such as mRNA can be efficiently sequestered.

The transfer of mRNA into eukaryotic cells by the techniques which have been previously used either involve the tedious manipulation of direct microinjection¹⁻⁸ or fail to protect the administered message from external nuclease degradation⁹⁻¹². Furthermore, entrapment of mRNA within erythrocyte ghosts, although overcoming the above mentioned problems, requires an external fusogen to be added to insert sequestered message into the cell. The use of liposomes enables encapsulated mRNA to be inserted into large numbers of cells, as well as cell types, without the addition of an added fusogen. Although the need for fusing agents may not be important when RNA is delivered to cells *in vitro*, it would preclude any potential *in vivo* application.

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Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes

SEVERAL interesting questions in developmental biology can be studied by the combination of messenger RNA from one kind of cell with the translational apparatus of another cell type. Such experiments can provide proof of the identity of a mRNA and can yield information regarding the species and cell-type specificities of translation systems. The first successful approach to this problem was that taken by Lane *et al.*¹ who injected globin mRNA into frog oocytes. However, results obtained using the oocyte, which is an unspecialised cell, cannot necessarily be extended to the translational apparatus of more specialised cells. Many workers have tried to introduce informational macromolecules directly into differentiated cells, using a variety of methods²⁻⁶. Stacey *et al.* have translated duck globin mRNA after injection into HeLa cells⁷. We have

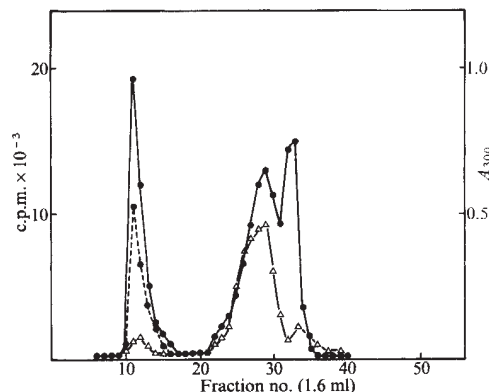


Fig. 1 Sepharose-4B chromatography of liposomes containing ¹²⁵I-globin mRNA. 10 μmol of beef brain phosphatidylserine in chloroform solution was evaporated to dryness under vacuum and suspended in NHTE buffer (0.1 M NaCl, 2 mM histidine, 2 mM triethylamino-ethanesulphonic acid, 0.4 mM EDTA, pH 7.4). The suspension was vortexed and subsequently sonicated under nitrogen in a bath-type sonicator at 30°C for 30 min. 0.02 mmol of Ca²⁺ was added and the mixture was then incubated for 1 h at 37°C after which the preparation was centrifuged at 2,500g for 10 min. The resulting pellet was suspended in 0.1 ml of a solution of globin mRNA (1 mg ml⁻¹) which had previously been dialysed against NHTE buffer, pH 7.4. In the experiment concerned with sequestering RNA into liposomes, trace amounts of ¹²⁵I-globin mRNA were added. The mixture of RNA and liposomes was vortexed, 0.2 mmol EDTA was added and the preparation was then incubated for 30 min at 37°C. The liposomes were recovered by centrifugation (30,000g, 20 min at 20°C) and washed with Ca²⁺, Mg²⁺-free PBS. Washed liposomes were resuspended in 1 ml of PBS, equilibrated for 30 min at room temperature and applied to a 1.2 × 42 cm Sepharose-4B column which had been equilibrated with the same buffer. Fractions (1.6 ml) were collected and A₃₀₀ (---△) and radioactivity (●—●) measured. The liposome fractions (void volume) were pooled and centrifuged at 30,000g for 20 min at 20°C. The resulting pellet was suspended in 1 ml PBS containing 1% Triton X-100 and incubated for 1 h at 37°C. After incubation the whole mixture was passed through the same column. △---△, radioactivity; ●—●, A₃₀₀ after treatment with Triton X-100. Isolation and iodination of rabbit globin mRNA was carried out as described elsewhere^{16,17}.

demonstrated^{8,9} the entrapment of ribonucleic acids of different sizes (28S, 18S, 9S and 4S) in large unilamellar liposomes. Such entrapped macromolecules are protected from ribonucleases and can be isolated both intact and biologically active⁸. Moreover, fusion of these liposomes yields RNA-filled cells⁹. We present here evidence that rabbit reticulocyte 9S mRNA introduced into mouse spleen lymphocytes directs the synthesis of globin.

We used large unilamellar liposomes, first prepared by Papahadjopoulos¹¹, which entrap high molecular weight molecules with high efficiency⁸⁻¹⁰. The process for the preparation of liposomes is described in the legend to Fig. 1. To separate vesicles containing RNA from untrapped RNA, the mixture of liposomes and RNA was passed through a Sepharose-4B column (Fig. 1). The fractions comprising the void volume were pooled and centrifuged at 30,000g for 20 min at 20 °C; the resulting pellet was resuspended in 2 ml of phosphate-buffered saline (PBS) and used for the translation experiments. To prove that the globin mRNA was really sequestered in the liposomal aqueous space, we incubated liposomes containing ¹²⁵I-globin mRNA in a solution of 1% Triton X-100 for 1 h at 37 °C (ref. 12). As shown in Fig. 1, after detergent treatment the ¹²⁵I-globin mRNA is retarded by the gel filtration column to the same extent as free globin mRNA.

For the translation experiments, purified mouse spleen lymphocytes were used. These cells were incubated for 2 h with liposomes containing globin mRNA and, after removal of liposomes, were incubated for a further 12 h with histidine-deficient Eagle's medium supplemented with 100 µCi of ³H-



Fig. 2 SDS-polyacrylamide gel electrophoresis of the immunoprecipitate from cells incubated with empty liposomes and free rabbit globin mRNA (a) and with liposomes containing rabbit globin mRNA (b). Spleen lymphocytes from BALB/c mouse were purified by using Ficoll-Hypaque²⁰. Antibodies against rabbit globin were prepared by immunisation of guinea pigs with purified rabbit globin (1 mg ml⁻¹) mixed with an equal volume of Freund's complete adjuvant. The immunisation procedures described by Avrameas were followed²¹. γ -globulin fractions were prepared from antisera by 40% ammonium sulphate precipitation and further purified by using immunoabsorbent of globin prepared with glutaraldehyde²². The purified antibodies gave a single arc in immunoelectrophoresis analysis and did not react with lysates prepared from mouse spleen lymphocytes. For the fusion of cells with liposomes, about 4×10^6 mouse spleen lymphocytes were incubated with 2 ml liposomes (2 µmol lipid per ml) as described in the text. For immunoprecipitation, 100 µl of cell lysates were used, following the method described by Craig *et al.*¹⁸. The immunoprecipitates were solubilised in SDS sample buffer and electrophoresed on a 12.5% polyacrylamide slab gel¹⁹. The gel was stained, dried and fluorographed.²³

histidine. The lymphocytes were then washed three times with Hanks' balanced salt solution, lysed with 1% Triton X-100 and then sonicated for 10 min. The cell debris was removed by centrifugation at 10,000g for 10 min, and 100 µl of the supernatant was removed for indirect immunoprecipitation¹⁸. Purified monospecific guinea pig anti-rabbit globin antibodies and goat anti-guinea pig IgG antibodies were used. The resulting immunoprecipitate was solubilised in SDS sample buffer and electrophoresed on a 12.5% polyacrylamide gel¹⁹. Control experiments were run exactly as described above, except that the lymphocytes were incubated with empty liposomes and free rabbit globin mRNA (20 µg ml⁻¹). Figure 2 shows the results of such an experiment: gel slot b shows that in cells incubated with liposomes containing rabbit globin mRNA, there is a band which co-migrates with globin; no such band of radioactivity is found in cells incubated with empty liposomes and free rabbit globin mRNA (slot a).

Our results show that mRNA for rabbit globin introduced into mouse spleen lymphocytes can be translated, giving rise to material closely related to rabbit globin. This finding contributes to the question of the cell-type and species specificities of the translation systems within the living cell. As rabbit globin mRNA is successfully translated in mouse spleen lymphocytes, we can conclude that all the components required to translate it are present in differentiated cells as different as rabbit reticulocytes and mouse lymphocytes. Thus, if messenger-specific components are required for the translation of globin messenger, then such components are present and available in mouse lymphocytes. If it is assumed that such factors do exist in mouse lymphocytes, then it is clear that their presence cannot be the only phenomenon determining the appearance of cell-type specific proteins during cell differentiation. Evidence for tissue specificity has only been found with purified cell-free extracts derived from terminally differentiated tissues^{13,14}. Of course, our results do not rule out tissue specificity for the different factors (such as initiation factors) because it can be argued that lymphocytes and reticulocytes have a common stem cell¹⁵. We believe that our system of introducing foreign mRNAs into differentiated cells by means of liposomes^{8,9} will give answers to such problems and will facilitate studies related to the synthesis and breakdown of mRNA in the host cell.

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matters arising

No evidence for a central serotonergic mechanism in arrhythmogenic effects of deslanoside

WE would like to comment on some of the data from this laboratory recently published in *Nature*¹. There is an error in Table 2 of ref. 1 involving the effects of 5,7-dihydroxytryptamine (5,7-DHT) on the 5-hydroxytryptamine (5-HT) concentration of the medulla-pons, hypothalamus, and colliculi of five cats. The data in Table 2 indicate that 5,7-DHT administered as a single dose of either 100 µg per kg to three cats, or 200 µg per kg to two cats, into the anterior horn of the left lateral ventricle 8 d before intoxication with deslanoside produces significant depletion of 5-HT in each of the three brain areas. Brain areas of seven cats were in fact examined but only data from five animals showed 5-HT depletion, and these data were presented in Table 2. The mean doses of deslanoside required to produce ventricular arrhythmia and ventricular tachycardia (but not ventricular fibrillation) in these five cats were significantly higher than corresponding doses in control animals. Calculation of the doses of deslanoside required to reach these same endpoints in all seven animals revealed no difference between the 5,7-DHT animals when compared with their controls.

The values for the brain 5-HT content of the 5,7-DHT treated animals are in error. We have repeated these experiments and found in seven animals who received 5,7-DHT (200 µg per kg into the anterior horn of the left lateral ventricle 8 d before intoxication with deslanoside), no significant depletion of 5-HT and no change in the doses of deslanoside which produce ventricular arrhythmias. We did find that the 200 µg per kg dose did produce significant depletion of norepinephrine in the hypothalamus. In addition, we have administered a much larger dose of 5,7-DHT (2.0 mg) into the lateral ventricle plus desmethylinipramine intraperitoneally to prevent 5,7-DHT uptake into noradrenergic nerve endings. This treatment resulted in a significant depletion of brain 5-HT but no significant alteration in the dose of deslanoside to produce ventricular arrhythmias.

In conclusion, we have no evidence for a role of central serotonergic mechanisms in the arrhythmogenic effects of deslanoside.

However, we are continuing to obtain evidence that a serotonergic mechanism is involved in the arrhythmogenic action of digitalis. Our current data indicate that it is 5-HT located in peripheral tissues and neural tissue outside the blood-brain barrier which may be important².

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Repressed motor nerve terminals in normal frog skeletal muscle

GRINNELL ET AL.¹ have demonstrated that when allowing two foreign nerves to innervate a transplanted frog sartorius muscle, control of transmission at neuromuscular junctions on muscle fibres is shared between the two nerves, one being more effective in terms of the quantal content of endplate potentials than the other. However, we previously² presented evidence of mutual repression by some axons in muscles with normal innervation. Grinnell *et al.*³ emphasise both the “quantitative and probable qualitative difference” between their results and ours, and they suggest that one possible explanation for the apparent extensive polynuclear innervation reported by us (up to 60% of the tension of one of the sharing motor units) is the low Ca²⁺ concentration used by us, 1.08 mM as against their 1.8 mM. Grinnell *et al.* agree with our findings of little or no detectable overlap in a comparison of twitch tensions, and are only challenging our estimates of overlap using tetanic tension. They put the upper limit of tetanic tension overlap at 25%, and in experiments using 1.08 mM Ca²⁺ they found a significant drop in twitch tension but no difference in the 50 per s tetanus tension. It seems to us that if there is no difference in the measured tetanic tension at the two Ca²⁺ concentrations, then other explanations must be sought to

account for the differences in amounts of overlap reported by Grinnell *et al.* and ourselves.

We agree that 1.08 mM Ca²⁺ is lower than is usually used, and since at this concentration neuromuscular transmission may begin to be affected (see, for example, ref. 4) we have repeated some of our experiments on sartorius using 1.8 mM Ca²⁺. None of the data (from 27 units in four experiments) differ significantly from those previously published by us, including both the value of twitch tension and the measurement of overlap. We therefore conclude that the differences in estimated overlap, as measured by us and by Grinnell *et al.* must either be due to the different species of frog used, or because Grinnell *et al.* did not make comparisons between single motor units, but were stimulating bundles of motor axons. We have shown (Fig. 5 in ref. 3) that overlap between motor units is not distributed uniformly throughout the population of units.

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GRINNELL ET AL. REPLY—We find it surprising that there is no difference between the twitch tensions in 1.08 and 1.8 mM Ca²⁺ (ref. 1), since in both *R. catesbeiana* and *R. pipiens* sartorius twitch tension changes sharply with such a Ca²⁺ change. However, accepting the findings of Luff and Proske we agree that the most likely explanation for the difference in our results is a species difference. That junctions in two different muscles can have very different safety factors is shown by the consistent difference we have observed between the sartorius and cutaneous pectoris muscles in the same species (A. G. & A. Herrera, unpublished). Presumably there could be equally large differences between the homologous muscles in two different genera of frogs.

The implication of Luff and Proske's finding is that in *Litoria aurea* overlapping junctions are either suprathreshold at 1.08 mM Ca²⁺, or so far below threshold that an insignificant number, if

any, reach threshold at 1.8 mM Ca^{2+} . However, they can all be driven to threshold by tetanic stimulation. This may indeed represent a form of competitive interaction between axons of the same nerve. In fact, it is apparently a more uniformly strong form of synaptic repression than we observe between pairs of foreign nerves.

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X-ray dose fractionation and oncogenic transformations in cultured mouse embryo cells

I AGREE with the main conclusion of Miller and Hall¹ that it is unwise to use data from experiments using single high doses of a carcinogen to estimate the likely effects of chronic low doses. However, in presenting their data they have used an inappropriate statistical technique which has in some cases considerably overestimated the accuracy of the observations which they present to support this conclusion. For example, for cells irradiated once with 50 rad they estimate the standard error of their observed transformation frequency per million surviving cells to be ± 4 , whereas in fact, it should be about ± 44 and possibly appreciably more.

The discrepancy arises as follows. They reported two experiments at 50 rad. In the first they observed a transformation frequency per million survivors of 137 (based on discovering a total of just four transformed colonies among some tens of thousands of survivors) and in the second they observed a transformation frequency of 130 (based on discovering just five colonies). Their final estimate of the transformation frequency per million survivors is 133 ± 4 , 133 being the simple average of 137 and 130 and ± 4 being based only on the coincidental similarity of the observed values 137 and 130, and taking no account of how these values arose. However, it is appropriate when

estimating standard errors from such data to bear in mind that only nine colonies have been discovered altogether, and as the 95% confidence interval corresponding to an observed count of nine is 4–17, the 95% confidence interval for the transformation frequency per million survivors should be at least as wide as 60–250 (and possibly appreciably wider, if other sources of error could be allowed for). If correct confidence intervals had been plotted in their Fig. 2 the surprising lack of any trend whatever in the split-dose data between 20 and 300 rad would be less definitely anomalous. (In addition, a minor improvement in data management would have been to use, instead of the average of the observed rates, the sum of the numbers of transformed colonies divided by the sum of the numbers of survivors.)

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MILLER AND HALL REPLY—Peto's comment on our paper¹ is fair and justified. The statistical analysis of data from transformation experiments of mammoth proportions, which yield only a half dozen or so transformed colonies from tens of thousands of irradiated cells, is a difficult problem. In retrospect, we should have quoted the standard errors based on Poisson statistics of the number of colonies observed, as well as the standard error calculated from the repeat experiments. In essence, Peto's point is that the closeness with which the absolute level of the transformation rate repeated between experiments was fortuitous, and on statistical grounds he is correct.

The main purpose of the experiments described, however, was to show that split doses produce more transformations than a single exposure of the same total dose. This conclusion still holds however the data are analysed. In experiments of this kind, the greatest reliance can be placed on comparisons within a given experiment, and in every case a significantly greater number of transformed colonies arise from split than from single doses. To establish the shape of the dose-response curve, much larger experiments would be necessary in which, preferably, several doses are used within the same experiment. This is not feasible for logistical reasons.

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Age of earliest mid-latitude glaciation

SHACKLETON AND OPDYKE¹ conclude, from oxygen isotopic analyses of core V28–179, that no large ice sheets accumulated in the Northern Hemisphere until about 3.2 Myr ago, shortly before the Mammoth event of the Gauss normal epoch. The core extends down into the upper Gilbert reversed epoch, and the bottom is estimated to be 3.6–3.5 Myr old; they conclude that from then until 3.2 Myr ago, Earth had a stable 'interglacial' or 'preglacial' climate. However, this is not true for the Southern Hemisphere. In South America the first recognised glaciation of Pleistocene severity occurred about 3.5 Myr ago, after the Cochiti event of the Gilbert reversed epoch; 50 km east of the Andean mountain front at about latitude 49°S, glacial till is interbedded with reversely-magnetised lava flows 3.55 ± 0.07 Myr old (above) and 3.48 ± 0.09 Myr old (below) (dated by the whole rock K Ar method)^{2,3}. This terrestrial evidence is consistent with some faunal analyses of Gilbert and Gauss-age cores obtained between latitudes 55°S and 63°S in the Southern Ocean, which show severe climatic conditions in the late Gilbert after the Cochiti event^{4–6}; sea ice may then have extended 1° to 1.5° north of its present position^{6,10,11}. If ice age cold prevailed in middle latitudes of the Southern Hemisphere 0.3 Myr before the initial formation of the Northern Hemisphere ice sheets, this would present an intriguing palaeoclimatic problem. It would suggest that these ice sheets could not form until after some further environmental change in the Northern Hemisphere: for example, uplift of the Labrador–Ungava Peninsula⁷, or final closure of the Central American seaway^{8,9}. More core analyses are evidently needed for a definitive reconstruction of global climatic and glacial events during the middle Pliocene.

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reviews

Purpose or chance?

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Purpose in a World of Chance: A Biologist's View. By W. H. Thorpe. Pp. 124. (Oxford University Press: London and Oxford, 1978.) £3.95.

PROFESSOR Thorpe knows as much as anyone else about animal language and this short book contains excellent discussions of it. He has also thought deeply about the philosophical problems of biology and gives a clear statement about these. I find myself in the main in agreement with his views about language but not with his philosophy, and probably many biologists will feel the same. This is not to say that we are right. Thorpe insists that in spite of all our knowledge of molecular biology and the action of the brain there remain great uncertainties, which it is only too easy to ignore. For him one of these "super problems" is the origin of life, the crux of which "is to envisage the origin of the cell". He believes that no biologist or physicist has been able to propose "even the outlines of a theory as to how such a cell might have 'evolved'". He thinks that "it may indeed have been a unique event, an event of zero probability". Obviously many biologists will not follow him here and will not agree that "the origin of life becomes an impenetrable barrier to science: a block which resists all attempts to reduce biology to chemistry and physics".

It is not entirely clear what grounds he has for this strong belief about the origin of life, but perhaps it is related to the solution he prefers for the other "super problem"—"The origin of Self-Conscious Mind". He is well aware of the difficulties of definition that this raises, and he carefully discusses how far it is of value to speak of mentality in animals. On such ethological questions he always has sensible things to say. For example, when he says "no-one has yet produced any convincing reason for believing animals to be aware of their mortality" one has confidence that he really has considered the evidence.

Although there is obviously a sort of scale of decreasing mentality he seems to reach the conclusion that in some sense mind is everywhere. He expresses this by extensive quotation from Whitehead to support the idea, under the caption that "The mental

(is) primary to the physical". He is ready to admit that Whitehead's metaphysics is opaque, but nevertheless castigates "the basic fatuity of the logical positivists", giving them no credit for calling our attention to the difficulties and restraints that language imposes on metaphysics. This distaste for logicians is a pity. It leads him to uncharacteristic scorn, where we should like to hear cool argument, of which he is so capable.

His defence of the purposefulness of living activities is most welcome, but is it necessary to associate it with particular views on the nature of mentality and a religious belief? These are questions that involve just that careful probing of words that linguistic analysis insists on. The concept of a "purpose" has long been central to Thorpe's philosophy and he defines it as more than the directed action of organisms

to survival. For him it is "a striving after a future goal retained as some kind of image or idea". With this definition he is inevitably committed to a dualism, which he believes to be the only logical and tenable position and which for him is associated with a Christian belief.

His views on these matters are nearly always well considered and interesting, whether one agrees with them or not. For my taste his book would have been better if he had given us more of them, with less quotation from his like-minded colleagues Eccles, Popper and Koestler(!). Let us hope that he will find an occasion to reiterate and amplify his faith in Whitehead's metaphysics, which is a matter of concern for all thinking scientists. □

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Haem metabolism

Heme and Hemoproteins. Edited by F. De Matteis and W. N. Aldridge. Pp. 449. (Springer: Berlin, Heidelberg and New York, 1978.) \$95; DM190.

THE simple title of this book may misleadingly discourage readers from a wider range of medical scientific disciplines than is deserved. The book is intended by its editors to assess critically a multi-disciplinary approach to haem metabolism by studies on patients, experimental animals and *in vitro* systems. It is concerned with the mechanism by which drugs and foreign chemicals disturb haem metabolism and the molecular events involved as shown by different biological responses which involve clinical medicine, genetics, toxicology, biochemistry and physiology as well as pharmacology.

The biosynthetic pathway of haem from glycine and succinyl-CoA by way of δ -aminolaevulinic acid (ALA), porphobilinogen, uroporphyrinogen III, coproporphyrinogen III and protoporphyrinogen IX, has long been known, although only recently have some details of the enzymic conversion of uroporphyrinogen to coproporphyrinogen and protoporphyrin become apparent. Haem synthesis to provide

for the synthesis and turnover of haem proteins is normally extremely efficient with loss of only minute quantities of intermediates; the control mechanisms responsible for this economy have been uncovered by the investigation of increased formation and loss of intermediates and the responsible enzyme defects in the genetically determined porphyrias in man and those in experimental porphyrias inducible by a variety of drugs. Haem itself exerts negative feedback control by repression of the rate-limiting enzyme ALA synthetase, and/or by decreasing its activity. A decreased amount or activity of an enzyme in the biosynthetic pathway would lead to haem deficiency unless corrected by the increased synthesis or activity of ALA synthetase.

Haem is utilised for the biosynthesis of haem proteins, but although these must be synthesised in all cells to provide for electron transport, peroxidase and catalase activities, there is a specially high requirement for haemoglobin synthesis in the bone marrow, myoglobin synthesis in muscle, and a less but equally important requirement in the liver (and rather less in the spleen and the kidney) for the biosynthesis of the microsomal cytochromes

P₄₅₀ and b₅. In these tissues there is at least one pool of free haem. Cytochrome P₄₅₀ with the flavoprotein cytochrome P₄₅₀ reductase constitutes the haem oxygenase system responsible for the degradation of the haem of haem proteins to bile pigments and for the biological hydroxylations of steroids and many drugs and foreign compounds, the administration of which induces an increased turnover of cytochrome P₄₅₀ leading to an increased requirement for haem. Whether it is the fall in free haem or cytochrome P₄₅₀ which increases ALA synthetase or its activity, is still obscure.

There is complex interaction between the enzymes of the haem biosynthetic pathway and the level and utilisation of the hepatic cytochromes and these interactions are affected by drugs in different ways.

There are few fields in which so much investigation has not led to a satisfactory synthesis of knowledge and the time is appropriate for an evaluation of the present situation. The contributions to the present work have clarified some of the discrepancies concerning the effects of drugs on the biosynthesis of haem and its incorporation into cytochrome P₄₅₀ as due to species differences, genetic differences and often different analytical techniques.

This useful volume consists of 11 contributions each with an excellent biography, two from one of the editors and the others from contributors of similarly high international status. G. H. Tait has reviewed the biosynthesis and degradation of haem and emphasises the need for enzyme purity for studies in this field. K. W. Bock and H. Remmer discuss the induction of cytochrome P₄₅₀ by many lipid-soluble drugs, insecticides and polycyclic hydrocarbons and conclude that cytochrome P₄₅₀ induction is a key process "in a complex chain of adaptive reversible events which lead to proliferation of endoplasmic reticulum membranes and to liver growth". T. R. Tephley summarises the effects of various substances, specially the herbicide aminotriazole and metallic ions in inhibiting haem and haem protein biosynthesis. F. de Matteis first attempts to elucidate the mode of action of substances causing loss of cytochrome P₄₅₀ by increasing its degradation, and then discusses the actions of porphyrin-inducing drugs which either cause a primary loss of haem synthetase to decrease haem synthesis and lead to an induction or activation of ALA synthetase, or cause a secondary non-specific effect by many lipid-soluble substances operating on a biological system already sensitised by the primary effect.

G. H. Elder has presented a comprehensive critical review of poisoning

by the fungicidal polyhalogenated aromatic hydrocarbons, especially hexachlorbenzene, and their mode of action; in common with lipid-soluble porphyrin-inducing drugs, they first increase hepatic cytochrome P₄₅₀ and later in certain species diminish uroporphyrinogen decarboxylase activity leading to a photosensitive porphyria. These effects are modified by many factors such as iron, oestrogen and perhaps liver damage. G. S. Marks has review in detail the pharmacokinetics of the porphyrin-inducing drugs in different experimental systems and discusses the relative merits of these for the investigation of the safety or otherwise of drugs likely to be given to porphyric patients. A. D. Maxwell and U. A. Meyer, also concerned with pharmacokinetics, emphasise the problem of drug idiosyncrasy due to genetic, hormonal and nutritional differences. The therapeutic effect of glucose in porphyria in blocking the induction of ALA synthetase, is considered by D. P. Tschudy, but remains unexplained. H. L. Rayner, B. A. Schacter and L. G. Israels in a comprehensive review (with nearly 600 references) of bilirubin metabolism examine in detail the abnormalities of each step from bilirubin formation to the faecal excretion of "urobilin", brought about by disease, drugs and other agents, including photolysis. Finally, S. Sassa has made an excellent contribution on lead toxicology in re-

lation to porphyrin and haem metabolism and emphasises the advantages of the measurement of blood protoporphyrin instead of urinary coproporphyrin or blood lead in the investigation of chronic lead poisoning.

This otherwise excellent work has not significantly considered haem and haem protein biosynthesis in systems other than bone marrow, liver and kidney, apart from enzyme deficiencies in the biosynthetic pathway in man recognisable in cultured fibroblasts obtained for diagnostic biopsy. Only two of the contributions refer to haem metabolism in the nervous system which is important for the elucidation of the neurological abnormalities in some of the porphyrias.

This book fulfils the ambitions of the editors but it is not a book to be read straight through; some overlap in the contributions frequently requires the reader to refer back to compare differences in experiment or interpretation. The editors might well have provided a critical summary as a final contribution. The subject index is good but the author index occupies over 50 pages instead of about 20 pages which a more orthodox index would require.

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Crystal structure index

Molecular Structures and Dimensions: Guide to the Literature, 1935-76. Organic and Organometallic Crystal Structures. Edited by O. Kennard, F. H. Allen and D. G. Watson. (Bohn, Scheltema and Holkema: Utrecht, for Cambridge Crystallographic Data Centre: Cambridge, 1978.) Df1.150;

THE study of crystal structures by diffraction methods has yielded details of molecular architecture of key importance for chemistry and biology. The scientific contributions of Bragg, Hodgkin and Perutz, for example, will be well known to readers of *Nature*. Because of developments in automated X-ray diffraction equipment, computers and methods of structure elucidation, there has been an enormous growth in the number of structural studies reported in recent years, and currently more than 2,000 references appear annually. Inevitably, even specialist crystallographers have difficulty in keeping track of the output of results in a wide range of journals.

To make diffraction results more readily available, Dr Kennard and her

colleagues at the Crystallographic Data Centre in Cambridge provide computer-based retrieval services for crystallographic results and edit a series of annual reference publications *Molecular Structure and Dimensions*. A brief description of the present volume is that it is analogous to a telephone directory. It consists of six comprehensive indexes containing bibliographical details of 15,933 organic and organometallic crystal-structure determinations published from 1935 to mid-1976. The indexes are derived from the compound names, molecular formulae, authors' names and literature references contained in volumes 1-8 of *Molecular Structure and Dimensions*.

The "Compound Name Index" is divided into two sections: the first for "Organic Compounds" and the second for "Organometallics and Metal Complexes". The organic index is presented as an alphabetical keyword listing and has 21,238 entries derived from 11,116 names. Special flags denote absolute configuration determinations and neutron diffraction studies. The organometallic and metal complex index lists 12,897 keywords derived from 6,464 names and here the entries are grouped under 54 metal-name subheadings. The "Molecular Formula Index" has 16,704

residues indexed according to their C,H content, and in the "Permuted Formula Index" the rarer elements are emphasised and 7,842 entries are grouped under 70 rare-element sub-headings. The "Author Index" has 43,039 citations to 10,362 authors ordered alphabetically by surname. The "Literature Index" has 15,933 citations from 260 journals, conference reports, and so on.

The "Compound Name Indexes" are valuable sources for the reader who wishes to discover what crystallographical results are available for particular types of organic or organometallic compounds. In the index of organometallic compounds and metal complexes, each subsection gives a comprehensive survey of crystallographic studies involving a particular metal and references to specific ligands can be obtained by working through the subsection. I quickly obtained 82 references to cobalt phosphine complexes, for example. In the organic index, I looked for bicyclo[3,3,1]nonane, cyclononane and cyclodecane derivatives and analogues and obtained 22, 6 and 30 references, respectively. A typical entry in this section has the form:

1,5-diaza-6,10- Cyclodecadione . . .
7 34. 8

where 7 specifies the MSD volume and 34 the compound class. On turning to the "Literature Index" the appropriate entry is:

7 34. 8 *Acta Crystallogr.*,
Sect.B . . . 31 1372 75

Here, 31 is the journal volume, 1372 is the initial page of the article and 75 is the year of publication (1975). Natural products with particular molecular features cannot normally be located by the keyword approach, as such compounds usually have trivial, rather than systematic, names. Accordingly, the list of cyclodecane derivatives and analogues did not include any germacranolide sesquiterpenoids and these have to be found by looking for particular compounds by name (for example, elephantol, glaucolide A, and so on) or by listing and examining all sesquiterpenoids (compound class 53).

The book is well produced and very reasonably priced for the wealth of information it provides in about 660 large pages (9 x 12 inches). It will be a welcome addition to libraries and laboratory desks. A minor flaw in the review copy is faulty printing of the final column in page L62 of the "Literature Index".

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Dream of classical thermodynamics

The Concepts and Logic of Classical Thermodynamics as a Theory of Heat Engines. By C. Truesdell and S. Bharatha. Pp. 151. (Springer: New York, Heidelberg and Berlin, 1978.) DM56.30; \$28.30.

In this book we pass from the elements of thermodynamics to the second law by methods and concepts which (the author assures us) were known in 1854, if only someone had had the insight to string the relevant ideas together. The mathematics is rigorous, and definitions, axioms, scholia, lemmas and corollaries abound. The text is furthermore spiced with critical and historical remarks regarding earlier treatments. Authors of textbooks in general are taken to task in no uncertain terms, for example, for not using the calculus (p. xv), or for appealing to "the absurdity" of perpetual motion machines "when reason fails" (p. xii). Now Truesdell is himself a considerable textbook writer if we recall *Thermodynamics for Beginners* (1966) and *Rational Thermodynamics* (1969), and it could be that he is here condemning his own earlier books, along with those

of others. One cannot be sure. The book is certainly more explicit about others: "In its hidden assumptions and vagueness, Sommerfeld's treatment is typical of those found in textbooks" (p49) or "Although . . . Clausius somehow fell upon the right answer, his reasoning is mainly of the physical or metaphysical kind and does not carry conviction to a critical student" (p147).

The night after I read the book I dreamt that I just entered a lofty hall which looked almost like a church. I recognised many people in the small congregation as textbook writers on thermodynamics from the present and the last century. A fiery speaker—clearly one of the authors—was just finishing an address "and thus our subject is a 'congenitally crippled science' (p.x), and (pointing his finger) you are to blame". There was agitation as he sat down and the Chairman, who I felt sure was Willard Gibbs, remarked in measured tones that a provocative speech, though not his way, was a stimulating experience. It fell to an elegant 1904 author, G. H. Bryan, to summarise the scientific content of the book. He pointed out that we are dealing with a system in two variables which, in a usual notation are v and t . We assume $p=p(v, t)$ and $(\delta p/\delta v)_t < 0$ (axiom I, p8); $dQ=C_v dt + l_v dv$ where $C_v > 0$ and l_v are appropriate functions

(axiom II, p30). The existence of a function $W(t_{hot}^{hot}, t_{cold}^{cold}, Q_{hot}(C)) > 0$ for the work done in Carnot cycle C is axiom III, p67, and that this function is the same for all bodies and invariant under a change of temperature unit gives the remaining axioms IV (p129) and V (p140). Normal thermodynamics can thus be constructed. "The historical remarks", he added with a smile, "mean that some extra space is needed." Thus, that $l_v = p = Nkt/v$ for an ideal gas is called Holtzmann's assumption, and that $C_p - C_v = Nk$ for such systems is called Mayer's assumption". In a brief discussion the chairman pointed out that the number of particles was clearly kept constant in the system, Nernst remarked that the absolute zero was not introduced, and Born pointed out that by having two variables with continuity assumptions the Pfaffian form for dQ has always an integrating factor so that some interesting thermodynamics was left out. In reply, the authors reiterated that their objective was to reach 1854, but to reach it with precision and historical footnotes. (There was a grumble from someone that footnotes for other than references was a sign of sloppy writing.)

Gibbs then introduced a discussion on the likely pedagogical success of the book. It was Sommerfeld's opinion that the mixture of mathematical arguments and sophisticated historical points concerning the thermodynamics of Carnot (itself a controversial problem), Clausius and Reech made the book hard for beginners. "The flouting of advice of international commissions on the use of scientific symbols makes it equally hard for those who know the subject", remarked Guggenheim. "What is one to make of the use of K_p and K_v not for compressibilities but for heat capacities?", he asked. "Are we then agreed it is useful for supplementary reading by young and old alike?", urged Gibbs. There was embarrassed silence. At last Lord Kelvin rose. "It is a contribution to our science, to which we have devoted our lives; it should be read and studied. But I would not like to think", he added with a twinkle, "that any young whipper-snapper can come along, do a bit of axiomatising of science and then look down his nose at those who expounded and created it before him. Generosity in science and in acknowledging indebtedness in science is a more important lesson to teach than entropy or energy". The applause turned into the low notes of my alarm clock, and I have written down here what I had dreamt. **P. T. Landsberg**

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Waves for beginners

Waves in Fluids. By James Lighthill. Pp. 504. (Cambridge University Press: Cambridge, New York and London, 1978.) £17.50.

THIS book is designed to provide an introduction to the theory of waves in fluids for students with little previous knowledge of fluid mechanics but with a sound background in elementary mechanics, ordinary differential equations and the theory of integral transforms. Its main thrust is to provide an understanding of their principal theoretical properties and to inculcate the ability to interpret the conclusions in a physical context. Readers of this book can expect to obtain a balanced view of the subject and quite a good basis for appreciating the advances now being made. The emphasis is on the linearised form of the equations but there are significant discussions of non-linear aspects as well.

It opens with an account of the simplest type of waves, namely sound waves, for which the dispersion relationship is linear and isotropic. Consequently, it is possible to explain in readily comprehensible terms such important concepts as sources, dipoles, radiation, scattering and dissipation. Next, the theory of one-dimensional waves is developed with application to a broad spectrum of topics in physics, including acoustics, distensible pipes and open channels. The crucial features common to the waves are that their characteristic length is short compared with that of the geometry of the propagation—for example, the variation in the shape of the channel and their amplitudes are small enough that non-linear effects develop slowly. By a succession of simple steps, clearly explained, we are brought to an appreciation of the physics of the formation of shocks and hydraulic jumps and of geometric acoustics.

In chapter 3 the theory of water waves is considered as an important example of dispersive systems in which the wave speed, or phase velocity, is a function of the wavelength. The chief message here is that now disturbances originating at some point at time $t=0$ propagate with the group velocity and not with the phase velocity. Many elementary textbooks founder at this point but (not surprisingly) the author sails through the difficulties with majestic ease. The student cannot fail to grasp the essential ideas, and now the way is open to the appreciation of many of the important features observed in wave motion.

Non-isotropic and even inhomogeneous dispersive wave motions are studied in the final and main chapter, with internal waves in stratified fluids

as a paradigm and we are led to the heart of the book—the discussion of ray theory, including caustics. This is a concept of great power and importance to scientists and engineers, who will value the lucid mathematical and physical explanations. The book ends with an epilogue, in part listing examples of other types of wave motion and in part explaining Whitham's theory of non-linear waves.

All students of fluid mechanics admire the author as an outstandingly successful contributor and expositor. The present book is an excellent example of his powers and will be especially appreciated by new converts to the field. Not only will they be able to learn the basic ideas of wave motions but also how to marry mathematics and physics in a mutually beneficial way;

and they can hone their technique on the large number of sensible and realistic examples included in the text.

From my reading of the book, and bearing in mind the aim of the author in writing it, I have happily found it impossible to formulate a criticism of any significance. The only regret is associated with the use of the word epilogue to describe the final chapter. I hope that this is not the author's farewell to wave motions in fluids and that he intends, or will allow himself to be persuaded, to write another volume giving us the benefit of his view of the modern state of the subject.

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Applications of catastrophe theory

Catastrophe Theory: The Revolutionary New Way of Understanding How Things Change. By A. Woodcock and M. Davis. Pp. 152. (E. P. Dutton: New York, 1978.)

THE aim of this book is to present catastrophe theory and its applications to readers untrained in mathematics or science. This is a difficult enterprise, not only because the mathematics underlying the main catastrophe classification theorem is extremely abstruse but also because the scope of application claimed for the theory is very wide, ranging from the buckling of beams through the development of the sea urchin's gut to the changes in a person's status on marrying out of his or her class. As well as all this, the authors describe the history of the theory's development, and assess the arguments in the current intense controversy about some of its applications.

In a short book, any treatment of these subjects must be superficial. But the public has a right to expect that what is written is at least correct, and I was sorry to find the book ruined by numerous errors of fact, misleading statements and evidence of careless haste in its compilation. It is not true that when a liquid boils its individual molecules undergo a transition, or that an umbilic catastrophe differs from a cuspid by involving a line jumping across a plane rather than a point jumping along a line, or that the conical shadow near the focus of a perfect lens is an envelope analogous to the caustic surface of an imperfect lens. The discussion of optics is illustrated by photographs with computer simulations that purport to correspond to them and in fact do not. The

discussion of buckling is illustrated with two figures, the second of which is incomprehensible without further explanation and is more confused by being carelessly interchanged with the first (the captions remaining where they should be). In the last diagram in the book (half intended as a joke) the behaviour axis is wrongly labelled. It should not be thought that such mistakes are somehow excusable when writing for non-technical readers; in fact, those who know nothing of a subject are more easily confused, and popularisers have a duty to be scrupulous in matters of fact and clarity of presentation.

The higher catastrophes are best illustrated by their projections on to the control space, but in this book such projections never appear. Even the cusp catastrophe projection is presented only once (in that same buckling diagram) and is inexcusably misdrawn with a corner at its singularity.

Much emphasis is placed on the theory's claim to (eventually) describe Nature's forms, but these are far too varied to be captured by one branch of mathematics. There is little mention of alternative possibilities, such as the numerology of leaf arrangement, the hierarchical 'fractal' structure of trees and blood vessels, the infinitely chaotic bifurcations implicit in population dynamics, and so on.

A colloquial style is used (we are told that Thom has a crew-cut, and Zeeman a bushy beard), implying not only that readers are non-technical but that their interest in the subject is not serious.

There is need for a popular exposition of catastrophe theory but this book does not satisfy it.

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